

Investigating the Role of Atmospheric Nitrogen Deposition and Biogeochemical Changes in Remote Cryospheric Environments

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M.Sc., Brown University: Providence, RI, 2017

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of
Philosophy in the Department of Earth, Environmental, and Planetary Sciences at Brown
University

Providence, Rhode Island

May 2020

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This dissertation by Sydney C. Clark is accepted in its present form by The Department of Earth, Environmental and Planetary Sciences as satisfying the dissertation requirement for the degree of Doctor of Philosophy.

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Curriculum Vitae

Education

- 2017, 2020 **M.Sc. and Ph.D. in Earth, Environmental and Planetary Sciences (Expected May 2020)**
Brown University, Providence, RI
- 2013 **B.S. in Aquatic and Fishery Sciences; Minor in Quantitative Science**
University of Washington, Seattle, WA

Professional Experience

- 2015–20 **Brown University.** PhD Candidate. Providence, RI.
- 2013–15 **University of Washington – Holtgrieve Ecosystem Ecology Lab.** Lab Manager. Seattle, WA.
- 2011–13 **Alaska Salmon Program.** Research Technician. Wood River, AK & Seattle, WA.
- 2012 **U.S. Fish & Wildlife Service Anchorage Field Office.** Summer Research Technician. Togiak, AK.
- 2011 **Bristol Bay Native Association & U.S. Fish and Wildlife Service.** Research Technician. Togiak, AK.

Research Experience & Relevant Skills

Field Experience

- Campaign logistics and project management, including permitting, sampling plans, volunteers, methodology, annual reports, equipment calibration/organization, data collection, transport, in-situ processing, documentation, motorized boat operation
- Remote field camp operations/fieldwork under a variety of conditions (e.g., AK, CO, OR, WA, Cambodia)
- Limnology measurements: physical (light attenuation, temperature, salinity), chemical (nutrients, pH, alkalinity, dissolved O₂), biological (plankton – phyto- and zoo-, chlorophyll-a), geological (some sediment smear slide analysis to assess lake history changes, diatoms as indicators of silica content, stable isotope sedimentary profiles -- $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ as indicators of changes in nutrient source and productivity)
- In-situ trace gas measurements (CO, CO₂, CH₄, N₂O, NO, NH₃)
- Sampling of different sample types: water, rainfall, snowpack, glacial meltwater and ice

Data Analysis & Software

- Matlab, R, Microsoft Excel, Word, Power Point, Illustrator

Analytical Experience (Concentrations, Stable Isotopes, Mass Spectrometry)

- Colorimetric Nutrient Analysis (Westco SmartChem): NO₃⁻, NO₂⁻, NH₄⁺
- Stable isotopes: $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$ of nitrate (Denitrifier method), $\delta^{15}\text{N}$ of ammonium (Azide method), $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$ of bulk tissue (combustion via EA-IRMS), $\delta^{18}\text{O}$ and δD of water (Picarro cavity-ring)
- Isotope Ratio Mass Spectrometry operation and trouble-shooting: GC-IRMS, EA-IRMS and Picarro Cavity-ring

Modeling Experience

- HYSPLIT (NOAA's atmospheric transport model)
- Isotope box modeling

Project Management, Teamwork & People Skills

Publications

Clark, S.C., J. Granger, A. Mastorakis, A. Aguilar-Islas, and M.G. Hastings. **2020**. An investigation into the origin of nitrate in Arctic sea ice. *Global Biogeochemical Cycles*. 34: e2019GB006279. doi:10.1029/2019GB006279.

Clark, S.C., R.A. Ryals, D.J. Miller, C.A. Mullen, D. Pan, M.A. Zondlo, A.A. Boateng, and M.G. Hastings. **2017**. Effluent gas flux characterization during pyrolysis of chicken manure. *Sustainable Chemistry and Engineering*. doi: 10.1021/acssuschemeng.7b00815.

Miller, D. J., P.K. Wojtal, **S.C. Clark**, and M.G. Hastings. **2017**. Vehicle NO_x emission plume isotopic signatures: Spatial variability across the eastern United States. *Journal Geophysical Research Atmospheres*. 122: 4698–4717. doi:[10.1002/2016JD025877](https://doi.org/10.1002/2016JD025877).

Pool, T., G. Holtgrieve, V. Elliott, K. McCann, B. McMeans, N. Rooney, A. Smits, T. Phanara, M. Cooperman, **S. Clark**, C. Phen, and S. Chhuoy. **2017**. Seasonal increases in fish trophic niche plasticity within a flood-pulse river ecosystem (Tonle Sap Lake, Cambodia). *Ecosphere* 8(7):e01881, doi: [10.1002/ecs2.1881](https://doi.org/10.1002/ecs2.1881).

Wojtal, P.K., D.J. Miller, M. O'Connor, **S.C. Clark**, and M.G. Hastings. **2016**. Automated, high-resolution mobile collection system for the nitrogen isotopic analysis of NO_x. *J. Vis. Exp.* (118): e54962. doi:10.3791/54962.

Clark, S.C., T.L. Tanner, S.A. Sethi, K.T. Bentley, and D.E. Schindler. **2015**. Migration timing of adult Chinook Salmon into the Togiak River watershed: Is there evidence for stock structure? *Transactions of the American Fisheries Society*: 144(4). doi:10.1080/00028487.2015.1031281.

Scientific Presentations

Clark, S.C., R.T. Barnes, I.A. Oleksy, E.F. Cerf, L. Gratz, J.S. Baron, and M.G. Hastings. Quantifying reactive nitrogen atmospheric inputs in an alpine system. American Geophysical Union Fall Meeting. San Francisco, CA. Dec. 2019 (*Poster presentation*).

Clark, S.C., R.T. Barnes, I.A. Oleksy, J.S. Baron, J. Orchardo, and M.G. Hastings. Quantifying reactive nitrogen atmospheric inputs in an alpine system. Brown University: Climate and Environment Lunch bunch. Providence, RI. Oct. 2019 (*Oral presentation*).

Clark, S.C., R.T. Barnes, I.A. Oleksy, J.S. Baron, and M.G. Hastings. Nutrient cycling in a warming alpine environment: investigating the provenance and persistence of atmospheric nitrate and ammonium deposition using isotopes. American Geophysical Union Fall Meeting. Washington, D.C. Dec. 2018 (*Oral presentation*).

Joyce, E., E. Le Roy, W. Walters, **S. Clark**, and M. Hastings. Highly concentrated nitrogen atmospheric deposition on an urban, coastal region. American Geophysical Union Fall Meeting. Washington, D.C. Dec. 2018. (*Poster presentation*).

Walters, W., M. Karod, **S. Clark**, E. Le Roy, D. Blum, E. Joyce, and M. Hastings. Seasonal, Spatial, and Temporal Investigations of Atmospheric Ammonia and Ammonium Dynamics in Providence, RI. American Geophysical Union Fall Meeting. Washington, D.C. Dec. 2018. (*Poster presentation*).

Clark, S.C., R.T. Barnes, I.A. Oleksy, J.S. Baron, and M.G. Hastings. Nutrient cycling in a warming alpine environment: An investigation of atmospheric nitrate and ammonium deposition using isotopes. Graduate Climate Conference, Pack Forest, WA. Nov. 2018 (*Poster presentation*).

Clark, S.C., J. Granger, A. Mastorakis, A. Aguilar-Islas, and M.G. Hastings. Arctic sea ice: an investigation into the origin of nitrate using $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$. Polar2018. Davos, Switzerland. June 2018 (*Poster presentation*).

Clark, S.C., J. Granger, A. Mastorakis, A. Aguilar-Islas, and M.G. Hastings. Arctic sea ice: an investigation into the origin of nitrate using $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$, International Symposium of Isotopomers. Baton Rouge, LA. Mar. 2018 (*Oral presentation*).

Clark, S.C., R.A. Ryals, D.J. Miller, C.A. Mullen, D. Pan, M.A. Zondlo, A.A. Boateng, and M.G. Hastings. Effluent gas flux characterization during pyrolysis of chicken manure. American Geophysical Union Fall Meeting. New Orleans, LA. Dec. 2017 (*Poster presentation*).

M.G. Hastings, **S.C. Clark**, J. Chai, E. DeFries, D.J. Miller, H. Schiebel, W.W. Walters, Tracking Reactive Nitrogen Sources, Chemistry and Deposition in Urban Environments with Stable Isotopes, American Geophysical Union Fall Meeting. New Orleans, LA. Dec. 2017 (*Invited talk*).

Clark, S.C., A. Mastorakis, J. Granger, A. Aguilar-Islas, and M.G. Hastings. Arctic sea ice: an investigation into the origin of nitrate using $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$. Brown University Climate and Environment Lunch Bunch. Providence, RI. Mar. 2017 (*Oral presentation*).

Clark, S.C., A. Mastorakis, J. Granger, A. Aguilar-Islas, and M.G. Hastings. Arctic sea ice: an investigation into the origin of nitrate using $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$. Association for the Sciences of Limnology and Oceanography. Honolulu, HI. Feb. 2017 (*Poster presentation*).

Clark, S.C., A. Mastorakis, J. Granger, A. Aguilar-Islas, and M.G. Hastings. Arctic sea ice: an investigation into the origin of nitrate using $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$. American Geophysical Union Fall Meeting. San Francisco, CA. Dec. 2016 (*Poster presentation*).

Clark, S.C. Chinook Salmon migration timing into the Togiak River watershed: Evidence of stock structure? Aquatic and Fishery Sciences Undergraduate Symposium. Seattle, WA. Mar. 2013 (*Oral presentation*).

Clark, S.C. Migration timing of adult Chinook Salmon into the Togiak River watershed: Is there evidence for stock structure? Alaska Salmon Program Research Symposium. Seattle, WA. Dec. 2012 (*Oral presentation*).

Teaching & Mentoring

Certifications

Sheridan Center Teaching Certificate Program (CERT I). Brown University. 2016-2017.

Teaching

GEOL 1150. Limnology. Dr. James Russell. Brown University. Spring 2017, 2019.

As a teaching assistant, I organized four fieldtrips, lead labs, graded problem sets, guest lectured, organized and conducted sample analysis for class final project, and designed a lab on graphics. Guest lectures included: *Phytoplankton and Zooplankton (2017)* and *Lake Morphometrics (2019)*.

GEOL 1950. Atmospheric Chemistry. Dr. Meredith Hastings. Brown University. Fall 2018

I gave a 90-minute guest lecture titled: *Oxidation Capacity of the Troposphere*

Mentoring

Emmie LeRoy, Felix Guo, Claire Bekker. 2019. Brown University. Undergraduate Lab Experience.

Emily Joyce. 2018. Brown University. Graduate Colorado Field Experience.

Celia Hack. 2018. Brown University. Undergraduate Lab Experience.

Fiona Cerf. 2017. Colorado College. Undergraduate Senior Thesis Reader.

Andrea Mastorakis. 2016. Brown University. Undergraduate Senior Thesis Mentor

Science Communication & Outreach

Science-A-Thon (October 13-17th, 2019, Brown University, Providence, RI)

Posted about my daily activities as a woman in science on social media as part of a fundraising campaign to raise awareness about women in STEM (proceeds went to organizations, such as ESWN and Girls Who Code)

Science Communication Essentials (September 20th, 2019, Brown University, Providence, RI)

Helped Jan Tullis organize department visit with MIT Curiosity Correspondent, Fatima Husain

Graduate Student Writing Workshop (Summer 2019, Brown University, Providence, RI)

Co-organized 4-part writing workshop with Emeritus faculty, Jan Tullis

Graduate Mentoring Program (2018-2020, Brown University, Providence, RI)

Help orient first year graduate students to grad school life by matching them with senior graduate mentors. The objective of this program is to ensure the new graduate students all have access to resources within the department to aid in their transition to the program.

Graduate Student Symposium (May 2016, 2017, August 2018, January 2020)

Procured departmental support and organized grad conferences where students could present work to peers, get feedback and practice communication skills in inclusive and supportive peer environment

Leadership Alliance Talk Panel (July 10th, 2018, Providence, RI)

Invited to provide feedback on talk presentations prior to program conference for students participating

Oral Presentation Skills Workshop Leader (August 7th, 2018, Boston, MA)

Helped international students practice Goldschmidt presentations

500 Women in Science at Providence College (January 23rd, 2017, Providence, RI)

Attended meet and greet with young female undergrads at PC interested in science

DEEPStep: Elementary Science Education Program (Summer 2017, Providence, RI)

Worked with a team of graduate students as part of DEEPStep Program to develop new science curriculum according to new district standards in summer 2017 for a 10-week unit for 4th grade.

Brown Science Prep (Spring 2017, Brown University, Providence, RI)

Worked with Nora Richter and BSP coordinators to construct a lesson about geologic records to teach Rhode Island high school students about the importance of geological records in our environment and what information they provide including

guest lecturers (Dr. Meredith Hastings and Nora Richter) to discuss the use of ice and sedimentary records in their research and an activity where students created their own geologic records, which would then be used to implement the creation of a science through art exhibit to be displayed in the Science Center of the Science Library at Brown University.

Professional Development Seminar: NSF-GRFP Workshop (Fall 2017, Brown University, Providence, RI)

Helped organize panel of professors (Chris Huber, James Russell) who had been NSF-GRFP panelists to give advice to GRFP applicants, developed peer-review groups for application materials and provided timelines to assist with curation of all materials prior to submission deadlines.

Vartan Elementary Science Conference (June 3rd, 2016 and 2017, Providence, RI)

Participated in science conference where taught 3rd graders about the salmon life cycle and how migration plays a large role in their sustainability with M.G. Hastings.

Vartan Elementary School Science Education (2016-2017, Providence, RI)

Taught science to 3rd graders two lessons a semester

Workshops

Effective Performance for Science Communicators (Oct-Nov 2019)

5-part series on effective performance strategies for scientists hosted by Trinity Rep Theater & Brown University

LaTeX (March 10th, 2019)

Short course in LaTeX hosted at Brown University

SPATIAL Short Course (June 11-23rd, 2017)

University of Utah - Salt Lake City, Utah

R-Workshop (January 20th, 2016)

One day workshop on utility of R Programing for diverse ecological applications hosted by University of Rhode Island & EPA Office, Narragansett, RI

Fellowships & Awards

Fellowships

- 2020 **Brown University.** Dissertation Completion Fellowship
- 2017-18 **Institute at Brown for Environment & Society.** Graduate Research Fellowship
- 2016-17 **National Science Foundation.** Grad Research Fellowship Program (*Honorable Mention*)
- 2015-16 **Brown University.** First Year Research Fellowship

Awards

- 2019 **Earth, Environmental and Planetary Sciences, Brown University.** GeoClub Service Award
Institute at Brown for Environment & Society. Graduate Research & Travel Grant
Brown University. Graduate Conference Travel Grant
National Geographic. Storytelling Explorer Grant. Contributor (*Unsuccessful*)
- 2018 **Brown University.** Graduate Conference Travel Grant
UW Graduate Climate Conference. Student Travel Grant
Earth, Environmental and Planetary Sciences, Brown University. Graduate Student Conference
Brown University. Doctoral Research Grant
Institute at Brown for Environment & Society. Graduate Research & Travel Grant
Brown University. Graduate School International Travel Grant
National Geographic. Early Career Grant (*Unsuccessful*).
- 2017 **Brown University.** Graduate Conference Travel Grant
Earth, Environmental and Planetary Sciences, Brown University. Graduate Student Conference
National Science Foundation. Stable Isotope (SPATIAL) Scholarship
- 2016 **Earth, Environmental and Planetary Sciences, Brown University.** Graduate Student Conference
Institute at Brown for Environment & Society. Graduate Research & Travel Grant

References

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- Colleague** **Joseph Orchard.** Instrumentation and Mass Spec Specialist,
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Aquatic and Fishery Sciences, University of Washington. *Seattle, WA, USA.*
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- Undergrad Advisor** **Dr. Daniel E. Schindler.** Professor,
Aquatic and Fishery Sciences, University of Washington. *Seattle, WA, USA.*
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Acknowledgements

First, I want to thank my Mom and Uncle Dick who would be tickled to know I made it through this marathon, but who are unable to celebrate with me at the finish line and motivate me to work hard every day. Thanks to all of my West Coast family for supporting my decision when I told them I was moving to Rhode Island for graduate school and always picking up the phone when I missed home.

Next, I would like to acknowledge all of the support, encouragement and valued mentorship I have received from my advisor, Meredith Hastings, who is a champion for all of her students and I feel lucky to have had the opportunity to work with her. I would also like to extend many thanks to my committee members: Karen Fischer and Jim Russell and outside readers: Warren Prell and Jill Baron for generously providing your time, advice and boosting my self-confidence in times of need. It has been a real pleasure learning from you and getting to share my work with you while at Brown.

Special thanks are in order to the Hastings Lab (Wendell, Jiajue, Emily, Dani) for coffee breaks and rigorous scientific discussions – I think of you all as family, Ruby Ho for always being generous with her time in and out of lab – I will never forget when you graciously helped me recover my car from my landlord's clutches after I thought it had been stolen during a snowstorm, Joe Orchard for spending copious amounts of time with me when I wanted more hands-on experience trouble-shooting and learning about our mass spectrometer, always reminding me to maintain a positive attitude and working with me on the dilution method – I have learned so much from you over these last 5 years, and finally Dorothy Fibiger who has been my big lab sister from afar.

Thanks to all of the folks who helped make my time on the east coast and in grad school truly memorable with heartfelt laughs, pot lucks, music sessions, game nights, softball, sailing and more. I am grateful to all of the softball enthusiasts (especially Bill, Tim, Brodie) who played with me on Billy's Blue Room, Softball Earth and on Team Garnet Gurus/Team Grad Students during past DEEPS Department Games; and the LONO sailing crew (Dave and Steve) for teaching me the ropes around a sail boat. I loved every game and race I have been able to participate in.

Finally, warm wishes and much appreciation for all of the folks who have helped bolster my morale during this adventure including: all DEEPS peeps, but especially a few (Jordyn, Nora, Abbie + Micah, Jenna + Brodie, my badass officemate: Sarah McGrath, Emily, Dani, Will Daniels, Marc Mayes), amazing friends near and far (Liz, Chloe, Alex, Kayla, Kaila, Adrienne + Todd, Ben), and my academic mentors and collaborators (Daniel Schindler, Thomas Pool, Arni Litt, Rebecca Barnes, Jill Baron, Bella Oleksy, Julie Granger, and especially Gordon Holtgrieve for continuing to send encouraging vibes).

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Chapter One

Introduction

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The work discussed in the following chapters seeks to understand the persistence of atmospheric nitrate and its role in reactive nitrogen biogeochemistry dynamics in ecosystems experiencing rapid environmental changes (i.e., a changing nitrogen depositional landscape and thawing cryosphere). The ecosystems in these studies include sea ice in the western Arctic Ocean and alpine mountain watersheds in the Rocky Mountains – both are limited by natural inputs of nitrogen and thus sensitive to external nutrient inputs. To explore and quantify the role of atmospheric nitrate in these remote ecosystems, I used a suite of field observations, analytical and experimental isotopic analyses, isotopic box modeling, long-term atmospheric transport and deposition chemistry trends. In sensitive ecosystems, the ability to quantify the extent and persistence of atmospheric reactive nitrogen deposition is critical for understanding connections between external contributions of reactive nitrogen and ecosystem biogeochemistry.

1. Nitrogen Biogeochemistry

Nitrogen (N) is essential for life, maintains the base of the food chain and is the most naturally abundant element in the atmosphere as dinitrogen (N_2) gas. Yet, few processes can naturally incorporate atmospheric N_2 into the environment, with the exception of lightning and biological fixation. N comes in oxidized and reduced forms, collectively referred to as reactive N (RN), which undergo transformations during movement between different environments. Biological fixation and denitrification naturally balance the incorporation of N_2 into the environment in RN forms (e.g., NO_3^- and NH_4^+) and the ultimate release of N_2 back to the atmosphere, respectively (Figure 1).

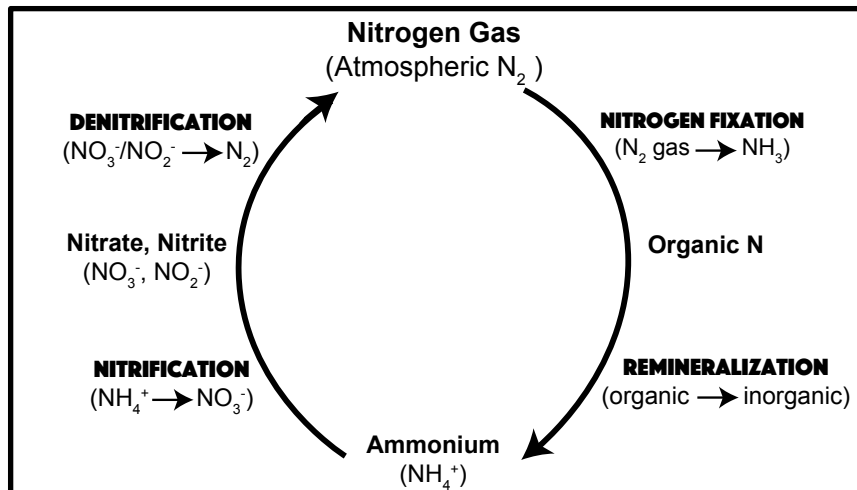


Figure 1: Simplified N cycle illustrating major processes resulting in incorporation of N₂ to the environment and release of N₂ back to the atmosphere. Major processes are in bold. Reactive N species (NH₃, NH₄⁺, NO₂⁻, NO₃⁻, organic N) produced as a result of these processes/transformations are identified.

However, anthropogenic RN sources, primarily originating from fossil fuel combustion and fertilizer synthesis and use, have caused an imbalance to the global N cycle – a problem dating back as early as the Industrial Revolution and turn of the 20th century to meet demands for energy and food production (Fowler et al., 2013). The compilation of impacts on air, land and aquatic systems induced with each additional RN atom in the environment before being returned as unreactive N₂ is referred to as the “Nitrogen Cascade” (Galloway et al., 2003). Natural sources of RN emissions include lightning, soil emissions (i.e., microbes), and biomass burning.

From an atmospheric perspective, gaseous forms of RN such as nitrogen oxides (NO_x = NO + NO₂) and ammonia (NH₃) are emitted into the atmosphere, chemically transformed, transported, and then deposited (as wet or dry deposition), distributing these species onto the landscape. The oxidation of NO_x eventually results in nitrate (NO₃⁻) and particulate-nitrate (p-NO₃⁻) deposition in wet forms of rain, snow and hail, or as dry deposition. Similarly, the neutralization of ammonia gas (NH₃) and particulate-ammonium (p-NH₄⁺) eventually deposit as NH₄⁺ in either wet or dry forms. Both NO₃⁻ and NH₄⁺ are important biologically and their abundance and accumulation in the environment results in a suite of ecological impacts including biodiversity losses, acidification of soils/surface waters and eutrophication of N-limited coastal

areas and lakes (Galloway et al., 2003; Hastings et al., 2013). The gaseous forms of RN also contribute to increased aerosol or particulate formation and ozone (O_3) production, impacting air quality and human health; and nitrous oxide (N_2O) released from enhanced biological processing (e.g., nitrification, denitrification) has implications for climate (Fiore et al., 2012; Palacin-Lizarbe et al., 2020).

In order to regulate RN emissions and deposition chemistry and reduce the impact on sensitive environments, it is important to understand the origin of source contributions and atmospheric chemistry processes that ultimately propagate the formation and deposition of RN that results in ecological impacts. From an atmospheric perspective, we seek to understand the influence of gaseous NO_x emissions, the chemical formation of nitric acid/nitrate (HNO_3 , i.e., nitrate (NO_3^-)) in the atmosphere, and the ultimate fate and distribution of NO_3^- into ecosystems experiencing rapid changes.

2. Atmospheric NO_x chemistry

Upon emission into the atmosphere, numerous chemical transformations can take place dictating the lifetimes of RN species such as NO_x ($=NO+NO_2$) (Jacob, 1999). The atmospheric lifetime of NO_x against deposition as NO_3^- is typically short, ranging from a few days up to a week.

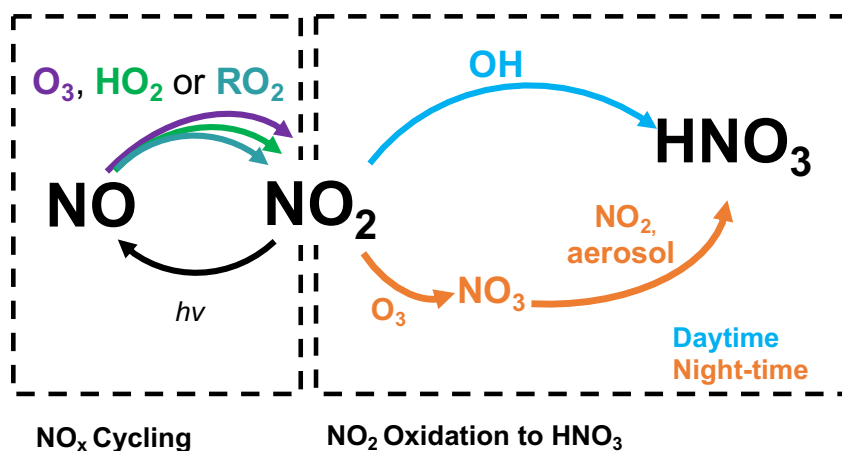
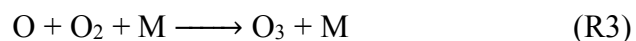
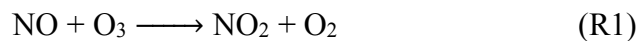
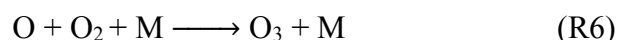
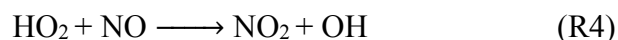


Figure 2: Formation of HNO_3 involves two primary oxidation steps: NO_x cycling and NO_2 oxidation to HNO_3 . Oxidation of NO to NO_2 occurs via 3 pathways (oxidation by O_3 , HO_2 , or RO_2). The oxidation of NO_2 to HNO_3 occurs via OH oxidation during the daytime or O_3 oxidation to form NO_3 , which reacts with NO_2 to form N_2O_5 and hydrolyzes on water vapor or aerosols (occurs at night). Adapted from Kunasek et al., 2008.

NO_x cycles rapidly between NO and NO₂ upon release in the atmosphere (Figure 2):

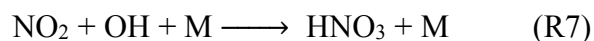


Reactions R1-R3 reflect the null cycle of O₃, allowing for rapid interconversion between NO and NO₂ during the daytime. Reactions R4-R6 reflect O₃ production and conservation of NO_x that takes place if NO₂ reacts with HO₂ and RO₂ atoms, although typically this is a minor pathway compared to NO oxidation via O₃ (Alexander et al., 2009):

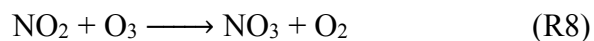


The oxidation of NO_x to HNO₃ is the major sink of atmospheric NO_x, which differs under light and dark conditions. During the daytime, formation of HNO₃ via OH oxidation is the dominant removal process of NO_x (R7), whereas O₃ chemistry dominates at night (R8-R10):

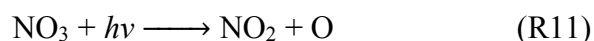
Daytime:



Nighttime:



This nighttime process (R8-R10) does not occur during the day because rapid photolysis of NO₃ would occur leading to NO₂ formation (R11):



These NO_x cycle reactions have a significant impact on the oxidizing capacity of the atmosphere, which determines the atmosphere's ability to remove various pollutants. Any HNO₃ formed via these reactions is readily deposited onto the landscape via wet or dry deposition, enhancing the accessibility (and environmental impact) of this desirable macro-nutrient.

3. Stable isotopic tracers of nitrate ($\Delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}$)

Precipitation chemistry, particularly in the U.S. and Europe, is monitored for concentrations of acidic species, such as HNO₃ (NO₃⁻ in dissolved form). However, NO₃⁻ concentration in precipitation alone does not exhibit clues regarding the initial NO_x source emissions or the atmospheric oxidation chemistry involved during the conversion of NO_x to HNO₃/NO₃⁻. Stable isotopes of N ($\delta^{15}\text{N}$) and oxygen (O) ($\delta^{18}\text{O}$, $\Delta^{17}\text{O}$) in NO₃⁻ present an opportunity for disentangling this information.

Two stable isotopes exist for N (¹⁴N and ¹⁵N), while O has three (¹⁶O, ¹⁷O and ¹⁸O), where the lightest forms (e.g., ¹⁴N and ¹⁶O) are the most naturally abundant relative to the heavier, less abundant (¹⁵N, ¹⁷O, ¹⁸O) forms. The relative abundance of these masses is presented in delta notation: $\delta = (R_{\text{sample}}/R_{\text{reference}} - 1) \times 1000\text{‰}$, where R is the ratio of the less abundant relative to the more abundant isotope (e.g., ¹⁵N/¹⁴N) and reference standards used for N and O include N₂ in air and Vienna Standard Mean Ocean Water (VSMOW), respectively.

The discovery that ozone (O₃) has an influence on the abundance of rare isotopes of O (¹⁷O and ¹⁸O) that is transferred to oxidized species during atmospheric photochemical reactions has been instrumental to advancing the field of atmospheric geochemistry (Clayton et al., 1973; Thiemens and Heidenreich, 1983). O₃ is an important oxidant in the atmosphere and has an elevated ¹⁷O and ¹⁸O composition relative to other oxidants (O₂, H₂O, OH, RO₂), which fall

along the mass-dependent, Terrestrial Fractionation Line (TFL: $\delta^{17}\text{O} = 0.52 \times \delta^{18}\text{O}$), defining the predictable fractionation behavior of terrestrial materials (Michalski et al., 2004; Figure 3). This deviation or anomaly describes the excess ^{17}O present in trace gases relative to ^{18}O and is defined as $\Delta^{17}\text{O}$ ($\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O} \neq 0\text{‰}$). Consequently, the oxidation of NO_x via O_3 results in NO_3^- deposition that contains a ^{17}O anomaly (i.e., $\Delta^{17}\text{O} > 0\text{‰}$) and acts as a conservative tracer of this interaction and atmospheric source (Michalski et al., 2003). This unique tracer has made significant contributions to our understanding of atmospheric chemistry and oxidation capacity of the atmosphere in both field and modeling studies (Kunasek et al., 2008; Geng et al., 2014, 2017; Fibiger et al., 2013; Alexander et al., 2009, 2019), as well as, gaining insights into the fate of atmospherically oxidized NO_3^- in biogeochemical work (Michalski et al., 2004; Hundey et al., 2016; Bourgeois et al., 2018; Clark et al., 2020).

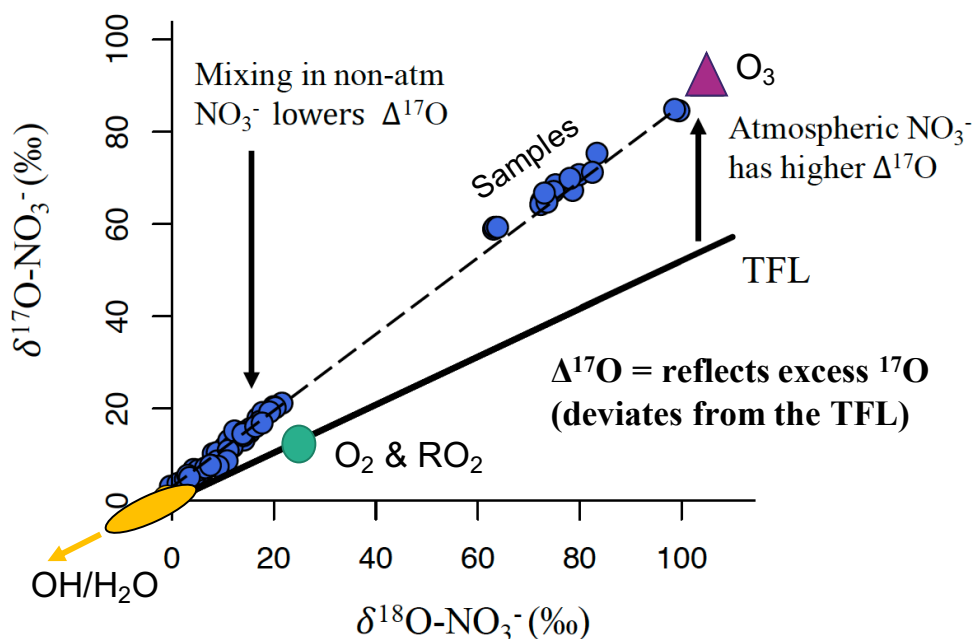


Figure 3: $\delta^{17}\text{O}$ vs $\delta^{18}\text{O}$ of nitrate illustrates the typical mass-dependent relationship ($\delta^{17}\text{O} = 0.52 \times \delta^{18}\text{O}$) defined by the terrestrial fractionation line (TFL, black line) and the deviation (dashed line) showing $\delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O}$ (or $\Delta^{17}\text{O}$). Atmospheric oxidants: O_3 , O_2 & RO_2 and OH & H_2O are reflected by purple triangle, green dot and yellow oval, respectively while samples are blue circles. Symbols that fall on the dashed line exhibit the excess ^{17}O anomaly (i.e., have a $\Delta^{17}\text{O} > 0\text{‰}$). Adapted from Michalski et al., 2004.

An interest in understanding the fate of atmospheric NO_3^- has stemmed from a legacy of atmospheric pollutants (resulting in acid rain) having a major impact on the ecological and biogeochemical functioning of ecosystems, as well as the development of isotopic tools to trace both source origins and fate within the environment. The complete isotopic composition of NO_3^- ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$) provides a wealth of information that can lend insight into NO_x sources and photochemical oxidation chemistry, as well as biological transformations within the environment (Figures 1-3). Recent studies have significantly improved our understanding of the $\delta^{15}\text{N}$ of specific NO_x sources (Fibiger and Hastings, 2016; Miller et al., 2017; Miller et al., 2018; Chai et al., 2019), which are thought to experience minimal fractionation during transformation to atmospheric NO_3^- prior to deposition. In addition, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ provide information specific to the oxidation pathways, which differ by season and atmospheric oxidants present (Michalski et al., 2012). Typical $\delta^{18}\text{O}$ of NO_3^- values range from 25 to 115‰ (Michalski et al., 2012 and references within) compared to biologically cycled NO_3^- ~0‰ in seawater (Casciotti et al., 2002). NO_3^- deposition in the mid- to high- latitudes has $\Delta^{17}\text{O}$ ranging between 22 - 40‰ (Michalski et al., 2012; Fibiger et al., 2013), compared to $\Delta^{17}\text{O} = 0$ ‰ for biologically produced NO_3^- . The production of NO_3^- from NH_4^+ oxidation (i.e., nitrification) adopts a $\delta^{18}\text{O}$ resembling ambient water (-1 to 1 ‰) (Sigman et al., 2009; Casciotti and Buchwald, 2012). During assimilation or denitrification, consumption of NO_3^- discriminates against heavy N and O isotopes equivalently and in proportion to NO_3^- consumption; this results in an increase in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- remaining (Granger et al., 2004, 2008). In contrast, $\Delta^{17}\text{O}$ of NO_3^- remains constant or unaffected during biological production and consumption because the fractionation is mass-dependent (i.e., $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ change mass-dependently). N and O isotopes thus provide complementary tracers to identify sources and cycling of NO_3^- . $\Delta^{17}\text{O}$ is an effective tracer of

atmospheric NO_3^- in ecosystems because any quantifiable $\Delta^{17}\text{O}$ composition indicates ‘uncycled’ atmospheric NO_3^- is residing in the system and as such, only non-atmospheric sources of NO_3^- (containing a $\Delta^{17}\text{O} = 0\text{‰}$) may act to dilute this high $\Delta^{17}\text{O}$ signature (Michalski et al., 2003).

The conservative behavior of the $\Delta^{17}\text{O}$ anomaly allows for the quantification of the percent of NO_3^- that originated from the atmosphere (Michalski et al., 2003; Michalski et al., 2004). In this dissertation, this quantification is referred to as percent atmospherically derived NO_3^- (%AD) and described by equation (1) below. This calculation can be conducted if the atmospheric composition has been well-constrained using atmospheric sample types (e.g., snow, aerosols or rain) and most often involves comparing the $\Delta^{17}\text{O}$ in a sample relative to a mean atmospheric value (Michalski et al., 2004).

$$(1) \%AD = (\Delta^{17}\text{O}_{\text{sample}} / \Delta^{17}\text{O}_{\text{atm sample}}) \times 100\%$$

A challenge commonly encountered with natural samples is the characterization of $\Delta^{17}\text{O}$ in low concentration samples. Current methods used for quantifying this anomaly have relatively significant sample size requirements (e.g., 50-300 nanomoles), and often require pre-treatment of the sample (e.g., resin column concentrating) to obtain a quantitative measure of this feature. Unfortunately, pre-treatment protocols often introduce matrix blank and fractionation effects that are not easily correctable and are challenging to conduct if sample volume is limited. To overcome this challenge, an alternative approach using isotope dilution was created (Chapter 3). Using this approach, samples are not pre-treated and samples with as little as 6-10 nanomoles can be analyzed with the aid of a reference spike in the sample solution with an uncertainty of $\pm 12\text{-}15\%$ or $2\text{-}3\text{‰}$. While this uncertainty is somewhat larger than typical methods, the lack of pre-treatment enables the analysis of low concentration and volume limited samples, which

otherwise may not have been possible at all. This method was developed during and implemented on alpine glacial samples from the Rocky Mountains (Chapter 5).

4. Sensitivity of ecosystems to external N sources

Alpine mountain and Arctic environments are rapidly changing due to a warming climate and thawing cryosphere (IPCC, 2019). Consequently, these naturally remote ecosystems represent harbingers of this change. The flow of nutrients associated with cryosphere changes, atmospheric deposition and surrounding terrain will be affected in environments experiencing these rapid changes, such as alpine lakes (Burpee and Saros, 2020), and Arctic Ocean environments. Remote alpine lakes are particularly sensitive to atmospheric inputs (especially N) resulting in altered biogeochemical cycles and algal taxa present as observed in paleolake records, as well as, present day monitoring programs (Moser et al., 2019; e.g., Baron et al., 2009). Similarly, sections of the western Arctic Ocean (e.g., Beaufort Gyre) are N-limited and sensitive to external inputs, but much less work has been done to study the impact of atmospheric N in the open Arctic Ocean or in Arctic sea ice. Reductions in sea ice extent and surface ocean warming in the Arctic Ocean have implications for surface primary production, polar fauna, shipping routes, and cultural fishing resources (IPCC, 2019). In order to understand a potential driver of change within these ecosystems, isotopic tracers of RN allow for the successful tracking of atmospheric NO_3^- in both systems (Chapters 2, 3 and 5), box modeling and mixing curve tools reveal insights on biological and physical dynamics in Arctic sea ice (Chapter 2), and precipitation chemistry and atmospheric transport modeling over time lend insight into whether transport changes dictate atmospheric trends that influence biogeochemistry in alpine surface waters (Chapter 4).

4.1 Fate of atmospheric nitrate in Arctic sea ice

Due to its proximity to industrialized emission sources, previous studies have suggested that NO_3^- in sea ice originates from the atmosphere in the Northern Hemisphere (e.g., Nomura et al., 2011). As sea ice continues to thin and sea ice distribution declines, the role of atmospheric NO_3^- in sea ice may become an important nutrient source in regions of the Arctic where macro-nutrients, such as N, are in short supply and high demand. Sea ice cores collected during a GEOTRACES Arctic Cruise are used to investigate the provenance of NO_3^- in late-summer Arctic sea ice (Clark et al., 2020). Indeed, atmospheric NO_3^- resides in all sea ice samples, but constitutes a surprisingly small proportion relative to NO_3^- that is microbially-produced from NH_4^+ (i.e., nitrified and making up $\geq 40\%$) (Chapter 2). Though further investigation is proposed, the role and possible significance of atmospheric NH_4^+ as a driver of this biological activity is discussed. These results suggest that atmospheric NO_3^- is not as important to the sea ice N budget as previously hypothesized (and potentially surface primary production), however additional constraints are needed to fully address the implications of sea ice melt as a budgetary perturbation to surface primary production in the Arctic Ocean.

4.2 Shifts in the reactive nitrogen deposition landscape

Since the implementation of the Clean Air Act and the National Atmospheric Deposition Program (NADP), precipitation chemistry has been heavily monitored across a vast network of monitoring locations. The development of the Clean Air Act has helped enforce stringent air quality regulations heavily focused on reducing precursory emissions of acid rain (SO_2 and NO_x). As a result, national trends in wet deposition of inorganic N have changed over broad spatial scales with dramatic decreases in NO_3^- (due to declines in NO_x emissions from industry

and vehicles) and steady increases in NH_4^+ (Du et al., 2014; Li et al., 2016; Lloret and Valiela, 2016). This shift is most apparent in the Northeastern and Midwestern regions of the U.S. (NADP, 2016), but is also occurring at smaller regional scales (Du et al., 2014), and suggests that the relative change of total inorganic N wet deposition has been steady or increasing since the implementation of these air emission policies (Schichtel et al., 2019).

Currently, NH_3 emissions are not federally regulated. Agricultural emissions continue to dominate the U.S. and global NH_3 budget (Warner et al., 2017). Newer vehicles are equipped with three-way catalytic converters intended to reduce NO_x emissions, but NH_3 is released as a byproduct upon inefficient NO_x conversion, thus vehicles have become a more important source of NH_3 in urban areas (Fenn et al., 2018; Li et al., 2016). As a result, enhanced NH_4^+ deposition trends have been suggested to be due to increasing urban NH_3 originating from vehicles and agricultural emissions from fertilizer-applications, livestock and feedlot waste (Fenn et al., 2018; Li et al., 2016; Fowler et al., 2013; Reis et al., 2009).

Emphasis has been placed on understanding trends across the national network, but it remains unclear whether the hypothesized sources are driving observed deposition trends at more regional and localized scales, such as in the Western United States, where dramatic changes in precipitation chemistry are not as easily apparent. A subset of NADP sites ($n=12$) are located at high elevations (>2500 meters) in Wyoming and Colorado and are in close proximity to large urban and agricultural sources to the east. These high elevation (i.e., alpine) sites are typically N-limited and thus sensitive to external loading of this desirable nutrient, which have numerous ecological impacts. Interestingly, NO_3^- concentrations in some surface waters at alpine sites (e.g., as observed in CO), remain elevated despite observed national declines in NO_3^- deposition.

Atmospheric N deposition is determined by emissions, chemistry, atmospheric transport, and deposition. Prior work has demonstrated the importance of seasonal meteorology and easterly upslope events delivering N to high elevation environments in the Western U.S. from localized sources in spring and summer (Beem et al., 2010; Benedict et al., 2013a-b; Benedict et al., 2018; Gebhart et al., 2011; Gebhart et al., 2014). Importantly, surface water NO_3^- concentrations in sensitive alpine ecosystems remain high, despite the national trend of decreasing NO_3^- deposition. It therefore remains unclear whether regional trends are reflective of the national trends, whether these trends are driven primarily by decreases in emissions (e.g., vehicles) as in other parts of the country or whether atmospheric transport pathways are also changing. Understanding these influences is critical to also then evaluating the fate and impact of NO_3^- deposition to alpine ecosystems.

In an attempt to constrain the relationship between deposition and surface water chemistry, long-term trends in annual and seasonal deposition chemistry and transport at an alpine NADP site in Colorado were assessed over a 20-year study period (1997-2016) (Chapter 4). Annual trends in NO_3^- and NH_4^+ concentration and deposition largely follow national observations, but the trends are not uniform across all western and alpine sites (Chapter 4). Concentration trends at one Colorado alpine site also exhibit annual declines in NO_3^- and stable or increasing trends in NH_4^+ while localized westerly atmospheric transport from UT/western CO becomes more important over this 20-year study period (Chapter 4). Similarly, seasonal concentration trends show the same general patterns for NO_3^- and NH_4^+ , however spring and summer concentrations tend to be higher and more variable.

While these results indicate local, westerly transport has become more important over time at annual scales, it is challenging to disentangle changes in concentration trends from

transport trends alone (event at seasonal scales) indicating that dominant seasonal trends are likely controlled by variable transport in addition to emission sources prevalent during specific seasons. Based on the annual transport results, additional regulatory measures of NO_x and NH_3 emission sources in western Colorado and northern Utah may be required moving forward if sensitive ecosystems, such as high elevation mountain watersheds, are to be effectively monitored and protected.

4.3 Fate of atmospheric nitrate in alpine watersheds

As discussed above, despite declines in atmospheric NO_3^- inputs, surface water NO_3^- concentrations remain elevated in alpine watersheds, but the source of the NO_3^- remains elusive. In the Rocky Mountains, where N is historically limited and rapid changes due to cryosphere thaw and atmospheric deposition are taking place, $\Delta^{17}\text{O}$ was used to quantify the proportion of atmospherically derived NO_3^- (%AD) in two Colorado alpine watersheds (Chapter 5). Field observations reflective of atmospheric (snow, rain), glacial (glacial ice, glacial meltwater) and surface water inputs were characterized for inorganic N concentrations (i.e., NO_3^- , NH_4^+ and NO_2^-) and the complete isotopic composition of NO_3^- ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$). The quantification of %AD was determined across the summer season in recent (2017-2018) and historic surface waters (mid-1990s, mid-2010s) in the Rocky Mountain alpine (Chapter 5). Atmospheric NO_3^- resides in these watersheds over space and time (mid-1990s, mid-2010s, 2017-2018) collectively spanning two watersheds and three decades, but it is a minor proportion of the budget relative to a microbially-produced source. The majority (80-89%) of surface water NO_3^- is microbially-cycled over the course of the summer season and may be driven by local increases in NH_4^+ deposition or organic matter remineralization from thawing permafrost/glaciers. If these trends

are driven by atmospheric NH_4^+ inputs, then atmospheric N deposition is still problematic and requires federal/state regulations of NH_3 emissions to mitigate future ecological impacts.

5. Implications

The proportion of atmospherically oxidized N within Arctic and alpine ecosystems was not a dominant portion of the N budget compared to a biologically-cycled source. NH_3 emissions continue to be unregulated, NH_4^+ deposition is increasing across national and regional scales, and likely makes an important contribution to NO_3^- dynamics in these systems – suggesting that inorganic N deposition is still having ecological impacts despite federal regulations on NO_x emissions. Increases in long-term, short-range westerly transport to a CO alpine site also suggest that monitoring/regulation of localized emission sources from the west should be considered when assessing reactive N budgets.

Future work in these areas should emphasize: whether increased melting of sea ice is an important perturbation to the N budget in the Arctic Ocean in order to constrain the impact on surface primary production across the entire basin, whether an adequate supply of remineralized organic matter released from glacial or permafrost thaw in alpine systems could explain/contribute to the elevated NO_3^- source present in surface waters and how that compares to incoming NH_4^+ deposition, and whether other N-limited ecosystems exhibit ecological impacts that could implicate increased NH_4^+ deposition due to lack of regulation on NH_3 emissions.

6. Summary of Appendices

Two investigations related to N inputs relevant for estuarine systems on the U.S. east coast are also included in this thesis. Appendix I (Clark et al., 2017) introduces the characterization of

carbon (CO , CO_2 , CH_4) and nitrogen (NO , N_2O , NH_3) gas effluent during the pyrolysis of chicken manure for biochar production. Results from this work have implications for assessing the sustainability of biochar production as an alternative and environmentally friendly soil amendment. Biochar has previously been shown to reduce gaseous emissions of greenhouse gases such as nitrous oxide. Because NH_3 emitted during the biochar production process can be high, the cost-benefit of using biochar at field scale was assessed for the Chesapeake Bay region, a hotspot of chicken manure production in the eastern U.S. Appendix II uses stable isotopes of bulk N in an estuarine sediment core to determine whether amendments in wastewater treatment practices are evident. Narragansett Bay, the largest estuary in New England suffers from poor water quality primarily due to excess N loading from sewage effluent. As a result, policies have focused on major reductions in wastewater effluent N via tertiary treatment at major wastewater treatment facilities along the Bay. Over the last decade, N loads to the Bay have decreased by ~50%, and this study (as part of a class project) was dedicated to understanding whether this change could be isotopically detected. The bulk N in the sediments integrates the biogeochemical response in the Bay, indicating that the biogeochemistry has indeed responded to the important nutrient source loading changes.

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Chapter Two

An investigation into the origin of nitrate in Arctic sea ice

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Published in *Global Biogeochemical Cycles* (2020), doi:10.1029/2019GB006279.

Abstract

Atmospheric deposition has been suggested to be an important source of reactive nitrogen stored in Northern Hemisphere land-fast ice, in contrast to Antarctic sea ice, where bulk nutrients originate predominantly from underlying seawater. A paucity of sea ice studies in the open Arctic Ocean limits our understanding of the potential for melting ice to contribute to primary production in N-deplete waters of the Arctic. As part of the U.S. western Arctic GEOTRACES 2015 expedition, samples of pack ice, overlying snow, atmospheric aerosols, and underlying seawater were collected between 82°N-89°N. To identify the provenance of N in sea ice, we measured a suite of tracers including the isotopic composition of nitrate, ammonium, water and particulate N. Relatively low concentrations of nitrate and ammonium were detected in sea ice (0.1-8.2 $\mu\text{mol L}^{-1}$ and 0.6-1.2 $\mu\text{mol L}^{-1}$, respectively), and in atmospheric samples (1.1-3.7 $\mu\text{mol L}^{-1}$ and 0.8-1.2 $\mu\text{mol L}^{-1}$, respectively). Atmospheric nitrate in snow had characteristically high $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ ($\Delta^{17}\text{O}_{\text{NO}_3} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O} = 27.1\text{-}33.5\text{‰}$ vs. VSMOW; $\delta^{18}\text{O}_{\text{NO}_3} = 70.8\text{-}87.8\text{‰}$), and low $\delta^{15}\text{N}_{\text{NO}_3}$ ($-5.9\text{-}2\text{‰}$ vs. N_2). In contrast to the atmospheric samples, the sea ice $\delta^{15}\text{N}_{\text{NO}_3}$ was typically higher ($-0.3\text{-}15.0\text{‰}$) and the $\delta^{17}\text{O}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ much lower ($\Delta^{17}\text{O}_{\text{NO}_3} = 0\text{-}12.4\text{‰}$; $\delta^{18}\text{O}_{\text{NO}_3} = 23.3\text{-}67.5\text{‰}$). The presence of $\Delta^{17}\text{O}_{\text{NO}_3}$ in the sea ice indicated 0-40% of the nitrate is sourced from the atmosphere, while the majority of the nitrate is non-atmospheric ($\Delta^{17}\text{O}_{\text{NO}_3} = 0\text{‰}$). Based upon concentration, isotopic observations, and dynamic box modeling with atmospheric deposition and biological processes, we find that the majority of nitrate can be explained by in-situ biological nitrate production.

1. Introduction

Nitrogen (N) is an essential nutrient whose availability modulates primary production in the ocean. Reactive N is delivered to the surface ocean from the subsurface by upwelling, and may also be introduced exogenously from rivers, biological N₂-fixation and atmospheric deposition. Nitrate, ammonium, and organic N deposited from the atmosphere originate from both anthropogenic and natural sources, though some estimates suggest that a vast majority of the N deposited to the world's oceans is anthropogenic in origin (Duce et al., 2008). In the western Arctic Ocean, reactive N at the subsurface is in short supply relative to phosphate and silicic acid, such that surface primary production is ultimately limited by the supply of N (Brown et al., 2015a; Tremblay and Gagnon, 2009). Atmospheric deposition of reactive N on sea ice and at the sea surface may thus represent an important source of reactive N fueling primary production (Tremblay et al., 2012), particularly in the oligotrophic Beaufort Gyre.

In the western Arctic, ice formation occurs in shelf polynyas, and floes are advected to the open basin by anticyclonic winds driven by the Beaufort High – a mechanism that helps to control the sea ice mass balance of the Arctic Ocean (Rigor et al., 2002; Moore et al., 2018). Pronounced declines in Arctic sea ice cover have resulted in increases in patchy, thinner, first-year ice in open water and sparser multiyear pack ice (Rosel et al. 2018; Hansen et al. 2013; Maslanik et al. 2011). As sea ice forms, cold, salty, dense brine is drained via brine channels due to gravity, which is replaced by warmer, fresher and less dense seawater that enters through porous sea ice (Wells et al., 2010; Worster and Rees Jones, 2015). Convective exchange between fresh seawater and salty brine is referred to as gravity drainage or brine convection, and is a prominent winter desalination process in sea ice that dampens ice permeability and also assists in nutrient replenishment (Notz and Worster, 2009; Vancoppenolle et al., 2010). Typically, gravity

drainage/convective exchange occurs at the ice-ocean interface during ice growth, but it can be triggered by warming at the ice surface (change in air temperature) and melt at the ice-water interface (change in ocean heat flux) which, when tested simultaneously, were found to produce deep convection that spanned the entire ice column (Griewank and Notz, 2013). This deep convection caused desalination that was most prominent in the lower half (*i.e.*, 40 cm) of the ice column due to enhanced brine upwelling and a stark temperature gradient between top and bottom ice resulting in strong salt advection and desalination in the lower half of the ice (Griewank and Notz, 2013). During the melt season in the Arctic, the reduction of snow cover can also result in surface water flushing of snowmelt causing desalination, where a high rate of brine removal is due to flushing of the upper ice by snowmelt and of the lower ice by a water layer formed from surface water runoff (Holt and Digby, 1985). Gravity drainage and flushing are considered to be the most prominent desalination mechanisms for winter and summer (Arctic only), respectively (Untersteiner, 1968; Notz and Worster, 2009). As the sea ice column warms, brine pockets become interconnected brine channels, which, along with ice melt, allow materials in sea ice to be released into surrounding seawater, including algal nutrients (Vancoppenolle et al., 2013).

In Antarctic sea ice, seawater surface nutrients are important for supporting/replenishing the cycling of N pools (Fripiat et al., 2014; 2015), with elevated nitrate concentrations ($>30 \mu\text{M}$) at the sea surface due to bottom-water upwelling. In contrast, surface seawater nitrate concentrations are depleted in the western Arctic ($<2 \mu\text{M}$) due to stark density contrasts in the nutrient-rich halocline of the upper water column—a function of freshwater inputs from surrounding landmasses, which suppresses nutrient upwelling from depth (Carmack et al., 2016 and references therein). Additionally, studies of land-fast ice in sub-polar regions in the Northern

Hemisphere (*i.e.*, the Baltic Sea and Sea of Okhotsk) suggest that atmospheric deposition can contribute significantly to reactive N concentrations (Nomura et al., 2011; Granskog and Kaartokallio, 2003; Kaartokallio, 2001; and Rahm et al., 1995). Nutrients and trace metals have been observed to be generally elevated in Arctic multiyear pack ice in the Fram Strait and Greenland Current relative to underlying seawater (Tovar-Sanchez et al., 2010). Sea ice also hosts diverse microbial communities within its matrix (Deming, 2010; Thomas et al., 2010), and a handful of Arctic (Rysgaard and Glud, 2004; Randall et al., 2012; Baer et al., 2014, 2015) and Antarctic studies suggest that sea ice has an active N cycle (Fripiat et al., 2014, 2015; Priscu et al., 1990).

Primary productivity has increased on the order of 20% in the shelf regions of the Arctic Ocean due to decreases in seasonal ice cover (Brown et al., 2015a.; Arrigo et al., 2008). Conversely, the open basins of the western Arctic have seen increased stratification and shoaling of the nutricline (McLaughlin and Carmack, 2010; Toole et al., 2010). This dynamic is potentially dampening primary production in ice-free regions and conferring increased influence of the exogenous reactive N supply on primary production with the increase in the length of the growing season. In the Northern Hemisphere, pollutant/nutrient sources from mid- to high-latitudes can be transported and deposited as wet or dry deposition onto sea ice and incorporated into the ice matrix (Nomura et al., 2011). The delivery of reactive N to surface waters from external sources such as melting ice could, therefore, become more important during the melt season (*i.e.*, late-summer). In other words, if reactive N in Arctic pack ice is primarily atmospheric, then it constitutes a net exogenous source of N to the ocean that could fuel new production in N-limited regions of the Arctic as the melt season progresses.

The provenance of reactive N in the environment can be identified from the naturally occurring stable isotopic ratios of different N species. In particular, stable isotopic tracers of nitrate ($\delta^{15}\text{N}$ vs. N_2 , $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ vs. Vienna Standard Mean Ocean Water (VSMOW)) present an ideal approach to distinguish atmospherically-derived nitrate from that produced biologically. (Delta is defined in units of per mil (‰) using the following: $\delta (\text{‰}) = [(R_{\text{sample}})/(R_{\text{standard}})-1] \times 1000$, where $R = ^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$ or $^{17}\text{O}/^{16}\text{O}$.) Atmospheric nitrate is highly enriched in ^{18}O and ^{17}O relative to other sources, with typical $\delta^{18}\text{O}$ values between 25 to 115‰ (Michalski et al., 2012, and references therein), compared to lower values (*e.g.*, $\sim 0\text{‰}$) for biologically cycled nitrate in seawater (*e.g.*, Casciotti et al., 2002, DiFiore et al., 2009). Moreover, atmospheric nitrate carries an anomalous “excess” of $\delta^{17}\text{O}$ relative to $\delta^{18}\text{O}$ due to interactions with atmospheric ozone, which is quantified as $\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O} \neq 0$ (Michalski et al., 2003). Nitrate deposition in the mid- to high- latitudes has $\Delta^{17}\text{O}$ ranging between 22 - 40‰ (Michalski et al., 2012, Fibiger et al., 2013), compared to $\Delta^{17}\text{O} = 0\text{‰}$ for biologically produced nitrate. The isotopic composition of nitrate is also sensitive to biological transformations. Nitrate produced biologically by nitrification (the oxidation of ammonium to nitrite then nitrate) adopts a $\delta^{18}\text{O}$ signature akin to ambient water (-1 to 1 ‰) (Sigman et al., 2009; Casciotti and Buchwald, 2012). Consumption of nitrate, from assimilation or denitrification, discriminates against heavy N and O isotopologues equivalently and in proportion to nitrate consumption, resulting in an increase in $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of nitrate remaining (Granger et al., 2004, 2008). The $\Delta^{17}\text{O}$ of nitrate, on the other hand, remains constant during biological production and consumption because the fractionation is mass-dependent (*i.e.*, $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ change mass-dependently). N and O isotopologues thus provide complementary tracers to identify sources and cycling of nitrate.

Using nutrient concentrations and isotopic observations (nitrate, ammonium, particulate nitrogen, water) in sea ice and atmospheric samples collected during the Arctic GEOTRACES 2015 cruise (USCGC HLY1502), we investigate the provenance of nitrate in pack ice between 82°N and 89°N and quantify the role of atmospheric nitrate. We then aim to understand and contextualize the full suite of isotopic observations in sea ice using mixing curve and box modeling approaches to explore physical mixing and biological mechanisms.

2. Methods

2.1 Sample Collection

Sea ice, snow, aerosols and seawater samples were collected during the 2015 GEOTRACES cruise on the USCGC Coast Guard Healy icebreaker from August 9th-October 12th, 2015 (referred to hereafter as late-summer). The cruise traveled from Dutch Harbor through the Bering and Chukchi Seas toward the north pole along the Makarov Basin and returned south via the Canada Basin in the Arctic Ocean. Sea ice cores (intact from the ice surface to ice-water interface, each 1-1.5 m in length) were collected at six sea ice stations (Fig. 1) between 82°N and 89°N in the Makarov and Canada Basins using a Kovacs Mark II corer (9 cm diameter). Cores were stored frozen onboard at -20°C, then subsampled in a walk-in freezer (-20°C) with a clean band saw and nitrate-free gloves at the UW-Isolab after arrival of the ship in Seattle, WA. Edges (~0.5 cm) were removed from the sides to minimize potential surface contamination. Cores were sectioned at 5 cm intervals for the top 30 cm, and ~10 cm thereafter, and transferred to pre-cleaned, high-density polyethylene (HDPE) bottles. In expectation of concentrations being extremely low, we wanted to minimize contamination as much as possible and thus ice samples were not filtered (see below). Discrete complementary cores from each station were also

sectioned to characterize physical parameters (Fig. S1), from which brine salinity and volume fraction (%) were derived (Eicken, 2003). Air temperature ranged from -12.34 to -1.17°C, snow cover was 100% at all stations and snow depth atop the ice ranged from 9.4 to 15.4 cm.

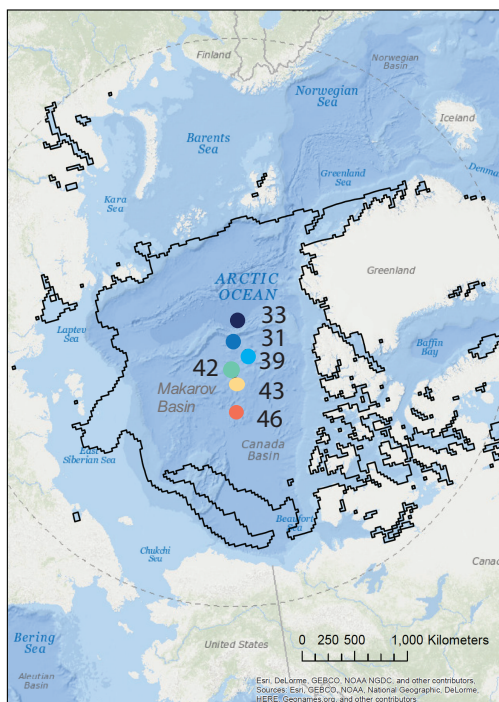


Figure 1: Sampling stations between 82°N and 89°N in the Canada and Makarov Basins during the 2015 GEOTRACES Arctic Cruise. Latitude and station numbers include: 89.96°N (33), 88.42°N (31), 87.78°N (39), 85.74°N (42), 85.16°N (43), and 82.49°N (46). Marker colors are used in figures throughout the manuscript to identify station locations of samples.

Aerosol collections took place over 3-5 day periods along the cruise track using pre-combusted glass fiber filters on high-volume samplers. Fourteen collections took place during the cruise, and triplicate samples for each collection were received. Extra deployment filters were collected with each set for blank characterization. Filters were kept frozen prior to being extracted in 30 mL of Milli-Q (ultrapure 18.2 Ω) water and filtered using 0.45 micron pore-size syringe filters.

Snow was collected from the ice surface at each station using a pre-cleaned shovel and transferred to a pre-cleaned polyethylene drum liner, which was sealed and transferred to the ship. Snow was then allowed to melt at room temperature. Once melted, snow samples were

filtered (Supor membrane, 0.2 μm pore size) and transferred to 250 mL pre-cleaned, HDPE bottles. Seawater was collected using Teflon coated Tygon tubing and a rotary pump with plastic wetted parts (IWAKI magnetic drive pump, model WMD-30LFY-115) from a hole at each station's sea ice floe. The hole was made with an ice corer (Kovacs 9 cm diameter Mark II corer), and allowed to sit undisturbed for ~ 1 hour under a canvas tent prior to sampling. Samples were filtered inline using Acropak 200 filter cartridges (Supor membrane, 0.2 μm pore size) from 1, 5, and 20 m below the sea ice.

Multiple ice cores were also taken at stations 39, 42, and 43 for particulate nitrogen (PN) analyses, where PN represents organic and bulk N particulates $>0.2\mu\text{m}$. For each station, the bottom 15-20 cm of four ice cores were combined and melted in filtered seawater, and then filtered onto a pre-combusted Whatman QMA quartz fiber filter (nominal 1 μm pore size) and dried at 60°C. Process and sorption blanks were obtained by passing previously filtered seawater through the QMA filters. All samples remained frozen until nutrient and isotopic analyses at Brown University, with the exception of the PN analyses, which were conducted at University of California Santa Cruz.

2.2 Nutrient Concentrations and Isotope Analyses

Nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) concentrations were measured using a colorimetric nutrient analyzer (Westco SmartChem 200), following standard protocols (based on USEPA Methods: 353.2 (Revision 2.0) for NO_3^- ; Method 354.1 for NO_2^- ; and Method 350.1 for NH_4^+). All samples were analyzed for NO_3^- (n=95: snow = 12, aerosols = 8, sea ice = 54, seawater = 16); a smaller set of snow (n=12), sea ice (n=28) and seawater (n=12) samples were also analyzed for NO_2^- and NH_4^+ . NH_4^+ was not analyzed in the aerosol samples due to sample volume limitations. Precision was calculated based on quality control replicates analyzed with

each sample set: $\pm 0.3 \mu\text{mol L}^{-1}$ for NO_3^- , $\pm 0.1 \mu\text{mol L}^{-1}$ for NO_2^- and $\pm 0.4 \mu\text{mol L}^{-1}$ for NH_4^+ , and detection limits were 0.3, 0.2 and $1 \mu\text{mol L}^{-1}$, respectively.

Aerosol samples ($n=42$) were extracted in 30 mL of Milli-Q water and sonicated for 1 hour. Concentrations were normalized for volume of air in cubic meters (m^3) passed through the filter during each collection (ranging from 96 to 452 m^3) and the volume of water for extraction to determine a final concentration in $\mu\text{mol L}^{-1}$ of air for comparison with non-aerosol samples. A number of aerosol sample extractions were found to be unfortunately contaminated with NO_3^- from a blank associated with the syringe filters used to filter the final solutions, and this data was discarded. Due to the discovery of this (inconsistent) blank, we chose not to filter the sea ice samples.

The N and O isotopic composition of NO_3^- ($\delta^{15}\text{N}_{\text{NO}_3}$ vs. N_2 in air, $\delta^{18}\text{O}_{\text{NO}_3}$ vs. VSMOW), was characterized using the bacterial denitrifier method (Sigman et al., 2001; Casciotti et al., 2002). For $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$, denitrifying bacteria (*Pseudomonas chlororaphis* spp. *aureofaciens* (ATCC® 13985™, Manassas, VA)) were used to quantitatively convert NO_3^- (20 nmol) to N_2O , which was then extracted, purified, cryo-focused and measured on a Gas-Chromatograph Isotope Ratio Mass Spectrometer (Thermo-Scientific Delta V Plus) in continuous flow mode. Samples were calibrated using internationally recognized NO_3^- reference materials (Böhlke et al., 2003 and references therein), including USGS34, IAEA-N3 and USGS35. Reproducibility for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ was calculated by taking the pooled standard deviation of sample and reference material replicates (Table S1). Precision based on sample replicates was $\pm 0.2\text{‰}$ and $\pm 0.4\text{‰}$ for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$, respectively ($n = 6$ pairs; Table S1). For all isotopic analyses (including $\Delta^{17}\text{O}_{\text{NO}_3}$), reference materials were prepared in Milli-Q water to bracket the concentration ranges of each sample run.

A separate aliquot of each sample was used to also determine the NO_3^- $\delta^{17}\text{O}$ composition ($\Delta^{17}\text{O}_{\text{NO}_3}$ vs. VSMOW) based upon decomposition of N_2O produced by the denitrifier method (Kaiser et al., 2007; Fibiger et al., 2013). The denitrifying bacteria (*Pseudomonas chlororaphis* spp. *aureofaciens*) were used to quantitatively convert 50 nmol of NO_3^- to N_2O , which is pyrolyzed to N_2 and O_2 using a gold tube heated to 770°C. $\Delta^{17}\text{O}_{\text{NO}_3}$ is then derived from the measured oxygen isotopic ratios ($\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O}$), and quantifies the mass-independent “excess” $^{17}\text{O}/^{16}\text{O}$ found in atmospheric trace gases that react with ozone (Kaiser et al., 2007; Thiemens and Heidenreich, 1983). Sea ice samples (n= 9) that did not meet the minimum concentration requirement for $\Delta^{17}\text{O}_{\text{NO}_3}$ analysis (<1 μM) were pre-concentrated using chloride ion resin exchange columns and 1M NaCl (Frey et al., 2009). For samples that required pre-concentration, standards were also pre-concentrated (see Shi et al., 2015). Reproducibility for $\Delta^{17}\text{O}_{\text{NO}_3}$ is ± 0.9 ‰ based on the pooled standard deviation of sample replicates (n = 4 pairs; Table S1). To quantitatively calculate the percentage of NO_3^- originating from atmospheric deposition in sea ice, the observed average atmospheric end-member (*i.e.*, snow) is used to represent $\Delta^{17}\text{O}_{\text{atm}}$ and the percent atmospheric deposition is calculated using: % AD = $\Delta^{17}\text{O}_{\text{sea-ice}}/\Delta^{17}\text{O}_{\text{atm}}$ based on Michalski et al. (2004).

Due to low sample concentrations and volumes, only 3 samples were analyzed for $\delta^{15}\text{N}_{\text{NH}_4}$ (snow=1; sea ice=2), using hypobromite oxidation to NO_2^- coupled with the denitrifier method (Felix et al., 2013; Zhang et al., 2007). Briefly, NH_4^+ is oxidized to NO_2^- with hypobromite, and NO_2^- is quantitatively converted to N_2O with denitrifying bacteria. For samples with sufficient analyte (n=3), oxidations were conducted in triplicate. Samples were calibrated using NH_4^+ reference materials USGS25 and IAEA-N2. The method precision is reported as ± 0.7 ‰ based on the standard deviation of reference replicates. Using the $\delta^{15}\text{N}$ - NO_3^- and - NH_4^+ ,

the $\delta^{15}\text{N}_{\text{NH}_4}$ is then computed by taking the weighted difference from measured $\delta^{15}\text{N}_{\text{NO}_3}$ to account for any NO_3^- (or NO_2^-) present in the sample (*i.e.*, the denitrifiers will convert all NO_3^- and NO_2^- present to N_2O).

Oxygen and hydrogen isotope ratios of water ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$ vs. VSMOW, $\delta\text{D}_{\text{H}_2\text{O}}$ vs. VSMOW) were measured using wavelength-scanned cavity-down ring spectroscopy on a Picarro L1102-I liquid analyzer calibrated to international reference standards: USGS46, USGS49 and VSMOW. Raw data were corrected for memory and drift effects followed by normalization using a standard calibration (Vaughn and Claymoore, INSTAAR, University of Colorado). Precision for $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta\text{D}_{\text{H}_2\text{O}}$ was better than $\pm 0.1\text{‰}$ and $\pm 0.4\text{‰}$, respectively, based upon repeated measurements of an internal standard (BW-1).

Particulate nitrogen (PN) and its stable isotopes ($\delta^{15}\text{N}_{\text{PN}}$ or $\delta^{15}\text{N}_{\text{PON}}$ vs. N_2 in air) from three sea ice stations were analyzed using a CE Instruments NC 2500 model Carbon/Nitrogen Analyzer interfaced to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (IRMS) at the Stable Isotope Laboratory at University of California, Santa Cruz. Samples with approximately 30-60 μg PN were fumed in a desiccator with concentrated HCl and dried in the oven at 60°C overnight, and then pelletized into tin discs. Samples derived from the IRMS were calibrated using Acetanilide as a reference material. Blank filters and tin discs were accounted for in the isotopic mass balance. The precision of the internal standard, Pugel, analyzed along with samples in the run was 0.2‰ for $\delta^{15}\text{N}$.

2.3 Isotopologue mixing curves

Concentration-dependent mixing curves using $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ as a conservative tracer were constructed between seawater and atmospheric NO_3^- end-members to test whether isotopic compositions retained in ice can be explained by simple end-member mixing (based on Fry,

2002). The seawater NO_3^- end-member values were presumed to reflect those of the subsurface NO_3^- reservoir in the open basin of the western Arctic, at 8‰, 0‰ and 0‰ for $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, and $\Delta^{17}\text{O}_{\text{NO}_3}$, respectively (Granger et al., 2011, 2018; Michalski et al., 2003; all non-atmospheric sources $\Delta^{17}\text{O}_{\text{NO}_3} = 0‰$). Three mixing curves were calculated using atmospheric end-members based on the minimum, maximum and mean observed isotopic values.

Isotopologue mixing curves are insensitive to end-member concentrations, thus mean observed values are used for both end-members. All end-member values used to calculate the mixing curves are shown in Table 1. Due to limited sample volumes, the aerosol extractions were not run for $\Delta^{17}\text{O}_{\text{NO}_3}$, and the aerosol data lack a relevant $\delta^{18}\text{O}_{\text{H}_2\text{O}}$. Given this and the fact that wet deposition (snow) is more representative (*i.e.*, contains both gas and soluble aerosol phase NO_3^-), we use the snow to represent the atmospheric end-members. However, mixing curves were also constructed with a combination of aerosol and snow observations to test if their combined isotopic composition changed the mixing curve results (Fig. S3). Mixing curves were also used to compare among isotopic properties ($\delta^{15}\text{N}_{\text{NO}_3}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$ vs $\delta^{15}\text{N}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$).

End-Member	$\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (‰)	$\delta^{15}\text{N}_{\text{NO}_3}$ (‰)	$\delta^{18}\text{O}_{\text{NO}_3}$ (‰)	$\Delta^{17}\text{O}_{\text{NO}_3}$ (‰)	NO_3^- (μM)
Seawater	-2	8.0	0.0	0.0	0.2
Snow Mean	-28*	-2.1	73.9	30.4	1.7*
Snow Min		-6.0	70.8	27.0	
Snow Max		2.0	87.8	35.0	

* $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ of snow (-28‰) and nitrate concentration (1.7 μM) are used for all mixing curves.

Table 1: Parameter values used to develop mixing curves between atmospheric deposition (AD) and seawater. Three mixing curves were created using values for the mean, minimum and maximum to cover the range in atmospheric deposition (AD) values for isotopes. Concentrations for each end-member and water isotopes were the only parameters held constant between curve scenarios (Fig. 5). Values for seawater end-member based on work by Granger et al. 2018; Fripiat et al. 2018.

2.4 Dynamic Box Modeling

2.4.1 Model construct

To investigate the potential influence of biological processing of atmospheric NO_3^- in sea ice, a time-dependent, box model was constructed to simulate the evolution of sea ice N (and O) isotopologues of NO_3^- , NH_4^+ and particulate organic nitrogen (PON) during biological cycling in sea ice. As a simplifying assumption, sea ice in the model is treated as a closed system. Nutrients and solutes from seawater and from atmospheric deposition are presumed to be enclosed in sea ice, and cycled therein from this initial condition. Admittedly, this closed-system assumption is invalidated upon brine rejection and exchange with underlying seawater in late spring and summer, which evidently occurred in the ice floes examined (see Results). Nevertheless, while brine loss and exchange with underlying seawater would expectedly dilute sea ice nitrogenous compounds with N-deplete seawater from the western Arctic Basin, these dynamics would not significantly modify the relationships amongst N isotopologue ratios. We thus exploit the box model to interpret the N isotopologue ratios and identify the provenance of the reactive N pools in sea ice while heeding the limitations of the model – which is not designed to reproduce N concentrations.

The governing model equations describe the assimilation of NH_4^+ and NO_3^- into PON that is subsequently ammonified and partially re-oxidized (nitrified) directly to NO_3^- along with representative ranges of associated fractionation factors (defined as $\alpha = \text{light}k/\text{heavy}k = (\varepsilon/1000) + 1$), where k values are the reaction rate constants for the light and heavy isotopologues, and ε is the kinetic isotope effect associated with a specific unidirectional reaction, as determined from field and laboratory studies (Fig. 2; Tables 2; see *S2: Box model of sea ice N dynamics* and Table S3-S4 for more details). We assume negligible accumulation of a NO_2^- intermediate, such that

the only N isotope effect associated with NH_4^+ oxidation in relation to the NH_4^+ pool, which influences the N isotope composition of NO_3^- produced by nitrification. The N isotope effect for NH_4^+ oxidation was prescribed based on the following empirical range (14 to 38‰), adopting a reference value of 19‰ (Casciotti et al., 2003; Fig. 2). The corresponding O isotope dynamics involve the assumption of full isotopic equilibration of the NO_2^- intermediate with water, with an equilibrium isotope effect of 13.5‰ (Casciotti et al., 2007; Buchwald and Casciotti, 2013), as well as isotopic discrimination of 15‰ for O-atom incorporation from water during NO_2^- oxidation (Buchwald and Casciotti, 2010). In turn, prescribed coupled N and O isotope effects for assimilation ranged from 5 to 10‰ (Granger et al., 2004; Difiore et al., 2009), adopting a reference isotope effect of 10‰, consistent with higher fractionation observed for light-limited NO_3^- assimilation (Needoba et al., 2003, 2004 and references therein). Corresponding $\delta^{17}\text{O}$ changes in a predictable way during mass-dependent biological transformations (*i.e.*, $\delta^{17}\text{O} = 0.52 \times \delta^{18}\text{O}$). The ammonification of PON to NH_4^+ has a small net isotope effect of $\leq 3\text{‰}$ (Lehmann et al., 2002), and here was assigned a nominal value of 0‰. Finally, isotopic discrimination during assimilation of NH_4^+ to PON is concentration dependent, ranging from negligible at relatively low concentrations to $\geq 20\text{‰}$ at concentrations $\geq 20 \mu\text{M}$ (Liu et al., 2013; Pennock et al., 1996; Hoch et al., 1992). We thus assign no isotope effect to this process, given the low observed concentrations ($\leq 1 \mu\text{M}$ NH_4^+). The isotopic signature of PN was assumed to reflect the isotopic composition of PON, and modeled $\delta^{15}\text{N}_{\text{PON}}$ was directly compared with PN observations. Denitrification is not represented in the model construct. Representative ranges of biological rates were simulated for assimilation, production and oxidation of NH_4^+ , as well as uptake of NO_3^- (Table S3).

Initial NO_3^- and NH_4^+ sources: atmospheric and shelf or seawater

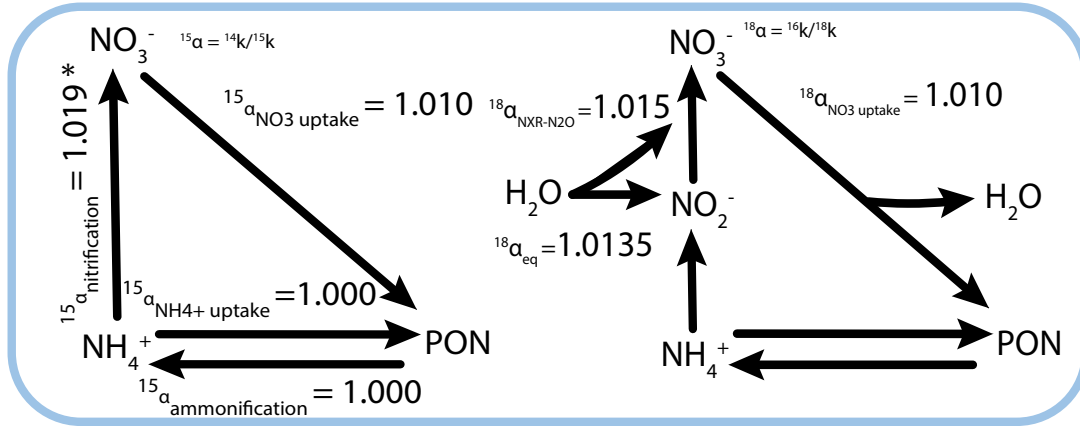


Figure 2: Model framework for biological processing of NO_3^- and NH_4^+ in sea ice with prediction of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$. Atmospheric deposition, shelf and/or seawater concentrations of nitrate and ammonium are used as initial boundary conditions in different scenarios described in Tables 2 and S3-S4. The asterisk (*) indicates that for *Scenario A*, this is the only isotope effect that is set differently from the other scenarios tested (*B-X*).

Model scenarios were initialized based on observed concentrations and isotopologue ratios of Arctic atmospheric and seawater end-members. Specifically, observed atmospheric NO_3^- and NH_4^+ values were used for the atmospheric end-member, while the seawater end-member reflects values representative of N-free seawater (*e.g.*, this study and Granger et al., 2018), and seawater on the western Arctic shelf (Granger et al., 2011; Brown et al., 2015b; Fripiat et al., 2018) (Table S4). Snow samples were used to represent the atmospheric end-member as it was more comprehensive and provided more data-points than the aerosol samples alone (*i.e.*, wet deposition provides an atmospheric column average versus the soluble aerosol extracts). For each model scenario, the time-dependent evolution of N isotopologue values in sea ice was also tested across representative parameter ranges, including isotope effects for $\text{NO}_3^-/\text{NH}_4^+$ assimilation and nitrification, and relative ratios of biological rates. Therein, a single parameter in the model was varied incrementally while keeping others at respective initial values (Fig. S5-S9). Specific details of these sensitivity analyses are described and tabulated in Supplementary Materials (Fig. S5-S9). Ultimately, the simulations of the time-dependent

evolution of N and O isotopologue ratios in sea ice, from initial conditions until steady-state, were quantitatively compared to observed ranges of $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$, $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ in order to gauge whether biological cycling within sea ice explains observed isotopologue distributions.

3. Results

3.1 Physical properties of the sea ice

Sea ice age estimates suggest that stations sampled between 87°N and 89°N consisted of first-year ice, while multiyear ice was sampled between 82°N and 85°N (M. Tschudi, NSIDC, 2015). Six-month back-trajectories suggest that ice originated from the East Siberian Arctic Shelf seas before being advected by transpolar drift to the Makarov Basin and the Beaufort Gyre (Kipp et al., 2018). Brine network connections occur when brine volumes exceed 5%, increasing sea ice permeability and fluid transport through porous sea ice (Golden et al., 1998). All ice cores appeared to have experienced brine loss where brine volumes/porosities were higher than 5% as a function of permeability (Fig. S1d; *S1: Ancillary Sea Ice Data*). The extremely low salinity above 20 cm is indicative of intense desalination, and therefore brine loss. As a result, flushing of salty brine by fresh surface meltwater percolating into pore space acts as an important desalination mechanism in the late Arctic summer (Untersteiner, 1968). However, brine drainage (*i.e.*, brine convection with underlying seawater) is also an important mechanism in Arctic and Antarctic sea ice generally (Griewank and Notz, 2013). Reflective of late-summer conditions, most of these cores exhibit desalination in an advanced stage, likely reflecting flushing of snowmelt water from the surface, which is exhibited in sea ice core isotopic properties.

The oxygen isotope composition of the sea ice ($\delta^{18}\text{O}_{\text{H}_2\text{O}}$) ranged between -18.6‰ and -0.8‰, suggesting that it derived from a mixture of seawater and meteoric water (Fig. 3a). Sea ice samples had $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ as low as -18.6‰ near-surface, though largely between -10.0‰ to -0.8‰, compared to characteristically negative $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values of -29.3 to -13.4‰ in overlying snow samples. Underlying seawater samples had a narrower range in $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, between -5.1 and -2.9‰ (Fig. 3a), with lower values likely reflecting a contribution of local sea ice melt, given a typical $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ of the mixed layer of ~ -2.5 ‰ in this region (Lansard et al., 2012). Four of the six cores (*i.e.*, salmon, beige, royal blue and navy) revealed a fairly uniform $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ composition from top to bottom (~ -4 ‰ on average), consistent with advanced desalination from flushing of meteoric water. The higher $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ in these cores relative to seawater may also reflect the preferential incorporation of ^{18}O to sea ice during freezing, which exhibits an enrichment of ~ 2 ‰ (Eicken, 1998; Melling and Moore, 1995). Using this average composition (-4 ‰), we can calculate that more than half of the sea ice cores were composed of ~ 18 % meteoric water and ~ 82 % seawater (meteoric water (snow) average = -22 ‰; seawater = 0 ‰). In contrast, two cores (cyan and turquoise) in the middle of the latitudinal transect exhibited a high percentage of meteoric water, in the top 50 cm (*i.e.*, ranging from ~ 45 to 82 %). This is indicative of mixing in of snowmelt, but only from the surface to ~ 50 cm for these two cores.

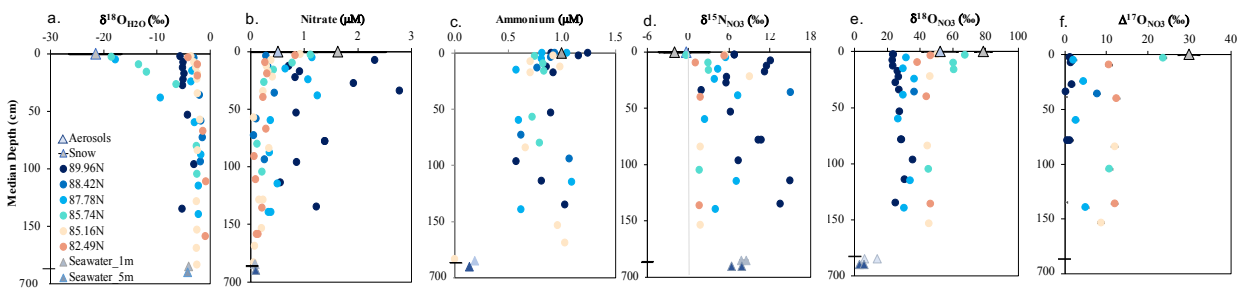


Figure 3: Profiles for a) $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (‰), b) nitrate (micromolar, μM), c) ammonium (micromolar, μM), d) $\delta^{15}\text{N}_{\text{NO}_3}$ (‰), e) $\delta^{18}\text{O}_{\text{NO}_3}$ (‰), and f) $\Delta^{17}\text{O}_{\text{NO}_3}$ (‰) against median depth (cm). Gray triangles represent the average value for snow; blue triangles are seawater values for samples collected at 1 and 5 m below the sea ice sample hole. Gray line in panel d) denotes the 0‰ line. Latitude for each station number includes: 89.96°N (33, dark navy circle), 88.42°N (31, royal blue circle), 87.78°N (39, dark cyan circle), 85.74°N (42, teal circle), 85.16°N (43, beige circle), and 82.49°N (46, salmon circle). Of note, one outlier for nitrate concentration ($8.2 \mu\text{M}$) is not shown in the concentration profile for the sea ice core sampled at 89.96°N .

3.2 Reactive N pools and isotopologues

In sea ice, the NO_3^- concentrations were relatively low in all samples, with mean values in snow of $1.7 \pm 0.8 \mu\text{mol L}^{-1}$ ($\pm 1\sigma$; $n=12$), $0.6 \pm 0.8 \mu\text{mol L}^{-1}$ ($n=12$) in aerosols, $0.9 \pm 1.2 \mu\text{mol L}^{-1}$ ($n=54$) in sea ice, and $0.3 \pm 0.5 \mu\text{mol L}^{-1}$ ($n=16$) in seawater (Fig. 3b). NO_3^- concentration profiles generally showed higher concentrations near/at the surface, which decreased with depth toward N-deplete seawater (Fig. 3b). This is consistent with previous observations from land-fast ice studies in the Northern Hemisphere (Granskog and Kaartokallio, 2003; Kaartokallio, 2001; Rahm et al., 1995; Nomura et al., 2011). Higher NO_3^- concentrations were observed at sea ice stations closest to the north pole (e.g. 89°N station, dark blue circles) than at stations further south (Fig. 3b), with a few sea ice samples exceeding concentrations found in snow samples (e.g. $8.2 \mu\text{mol L}^{-1}$). NO_2^- was below detection in all samples analyzed. NH_4^+ concentrations were similarly low, with mean snow NH_4^+ concentrations of $1.0 \pm 0.1 \mu\text{mol L}^{-1}$ ($n=12$), sea ice concentrations of $0.8 \pm 0.2 \mu\text{mol L}^{-1}$ ($n=28$), and seawater concentrations of $0.2 \pm 0.1 \mu\text{mol L}^{-1}$ ($n=12$) (Fig. 3c). NH_4^+ concentrations among sea ice cores did not exhibit distinct trends associated with depth or latitude. Collectively, NH_4^+ concentrations were comparable to those in overlying snow, yet elevated compared to underlying seawater (Fig. 3c). This is analogous to other observations where mean NH_4^+ concentrations in sea ice were high relative to underlying seawater in the Fram Strait and Greenland Current (Tovar-Sanchez et al., 2010).

Snow samples atop sea ice had isotopic values characteristic of atmospheric deposition with generally negative $\delta^{15}\text{N}_{\text{NO}_3}$ (-5.9 to 2.0‰), and high $\delta^{18}\text{O}_{\text{NO}_3}$ (70.8 to 87.8‰) and $\Delta^{17}\text{O}_{\text{NO}_3}$ (27.1 to 33.5‰ ; Fig. 3d-f, S2). Aerosols exhibited a similar range as snow samples, with slightly more positive values for $\delta^{15}\text{N}_{\text{NO}_3}$ (-3.6 to 5.9‰) and a larger range for $\delta^{18}\text{O}_{\text{NO}_3}$ (39.8 to 74.5‰), (Fig. 3d-e, S2a-b).

Compared to the atmospheric samples, sea ice samples tended toward higher $\delta^{15}\text{N}_{\text{NO}_3}$ (-0.3 to 15.0‰; Fig. 3d, S2a), whereas $\delta^{18}\text{O}_{\text{NO}_3}$ (23.3 to 67.5‰; Fig. 3e, S2b) and $\Delta^{17}\text{O}_{\text{NO}_3}$ (0 to 12.4‰; Fig. 3f, S2c) were intermediate between atmospheric values and a non-atmospheric source (*i.e.*, $\Delta^{17}\text{O}_{\text{NO}_3}=0\text{‰}$). No distinctive trends in sea ice $\delta^{15}\text{N}_{\text{NO}_3}$ profiles were observed. However, the northernmost ice station samples had higher NO_3^- concentrations and relatively higher $\delta^{15}\text{N}_{\text{NO}_3}$ (>8‰ throughout most of the profile; see Fig. 3d). Also, the northern-most core contained the lowest $\delta^{18}\text{O}_{\text{NO}_3}$ (~23‰) and $\Delta^{17}\text{O}_{\text{NO}_3}$ (~0 to 2‰) preserved in the ice (Fig. 3e-f). Cores collected furthest south (82°N-85°N) broadly exhibited lower $\delta^{15}\text{N}_{\text{NO}_3}$ values (surface values of <5‰), and higher $\delta^{18}\text{O}_{\text{NO}_3}$ (~38-65‰) and $\Delta^{17}\text{O}_{\text{NO}_3}$ (~8-23‰) values from top to bottom compared to the three more northern cores ($\delta^{18}\text{O}_{\text{NO}_3} = \sim 22\text{-}37\text{‰}$ and $\Delta^{17}\text{O}_{\text{NO}_3} = \sim 0\text{-}8\text{‰}$) (Fig. 3d-f). For the purposes of this study, sea ice cores were considered as a collective dataset and we focus on the range of the observations as representative of late-summer Arctic sea ice.

Seawater samples under the sea ice had relatively elevated $\delta^{15}\text{N}_{\text{NO}_3}$ (5.9 to 8.5‰), and the lowest $\delta^{18}\text{O}_{\text{NO}_3}$ range (0 to 13.8‰; Fig. 3d-e, S2a-b). $\delta^{15}\text{N}_{\text{NH}_4}$ analyzed in two snow samples had values of 3.0 and 3.9‰ at Stations 42 and 31, respectively, while a single measured sea ice sample had a positive $\delta^{15}\text{N}_{\text{NH}_4}$ of 11.7‰ at Station 39 (Table S2). $\delta^{15}\text{N}_{\text{PON}}$ (*i.e.*, $\delta^{15}\text{N}_{\text{PN}}$) measurements from sea ice stations 39, 42, and 43 ranged between 2.1 to 4.3‰ (n=3).

4. Discussion

To begin, we compare our isotopic results with previous studies and assess aspects of the results that are important for interpretation. The atmospheric samples are representative of expectations for NO_3^- formed in the atmosphere. Namely, atmospheric NO_3^- is high in $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ and relatively low in $\delta^{15}\text{N}_{\text{NO}_3}$, compared to other sources of NO_3^- . The high $\delta^{18}\text{O}_{\text{NO}_3}$

and $\Delta^{17}\text{O}_{\text{NO}_3}$ reflect the influence of ozone on the formation of NO_3^- in the atmosphere, and the values in this study should reflect the oxidation chemistry responsible for the formation of NO_3^- , which varies regionally (*e.g.*, Morin et al., 2011). There are limited observations of atmospheric NO_3^- concentrations and isotopes in the cruise region, and none from this region of the open ocean. Snow and aerosol samples collected at a station in Alert, Canada (Morin et al., 2008) and near the coast of Ny-Ålesund, Svalbard (Morin et al., 2009) share broadly comparable ranges for $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ (snow only) for atmospheric NO_3^- . However, in both studies, summertime $\delta^{15}\text{N}_{\text{NO}_3}$ exhibited very negative values – as low as -42‰ in filtered air from Alert, and -22‰ in snow near the coast of Svalbard. The very low $\delta^{15}\text{N}_{\text{NO}_3}$ values ($< -20\text{‰}$) in these studies are attributed to NO_3^- that is formed as a result of precursor nitrogen oxides photolytically released from snow-covered surfaces. This photolytic process leads to a very large isotopic fractionation (*e.g.*, Berhanu et al., 2015), such that the photolytically released nitrogen oxides have very negative $\delta^{15}\text{N}$ values, while the snow NO_3^- is left highly enriched in ^{15}N . Our open ocean-atmospheric observations do not exhibit these very low $\delta^{15}\text{N}_{\text{NO}_3}$ values, suggesting that recycled snow-sourced nitrogen oxides are not an important source. This may be the result of relatively fast recycling of nitrogen oxides to NO_3^- and re-deposition on the continents (Zatko et al., 2016) versus the open ocean. Additionally, the surface snow samples did not show any direct evidence of significant photolytic loss of NO_3^- , especially given that both the $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ were not significantly higher than what is observed in aerosol samples.

Currently, there are two sea ice NO_3^- isotopic studies, both from Antarctica, and none from the Arctic, with which to compare our results (Fripiat et al., 2014, 2015). In Antarctic spring pack ice cores collected at two drifting stations, a fairly narrow range was observed for $\delta^{15}\text{N}_{\text{NO}_3}$ (2.1 to 8.1‰ and 4.1 to 5.1‰, respectively), and $\delta^{18}\text{O}_{\text{NO}_3}$ was not measured/reported

(Fripiat et al., 2014). Fripiat et al. (2015) reported $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ as a time series in the evolution of sea ice in the southern hemisphere winter and spring for land-fast ice. In that study, the authors observed a narrow range in wintertime ice ($\delta^{15}\text{N}_{\text{NO}_3}$: $5.6 \pm 0.3\text{‰}$, $\delta^{18}\text{O}_{\text{NO}_3}$: $3.3 \pm 0.4\text{‰}$) and wider range in $\delta^{15}\text{N}_{\text{NO}_3}$ (6 to 20‰) and $\delta^{18}\text{O}_{\text{NO}_3}$ (5 to 20‰) in spring time-collected sea ice (Fripiat et al., 2015). While some overlap exists with the $\delta^{15}\text{N}_{\text{NO}_3}$ values observed in the late-summer Arctic sea ice profiles from this study, all of the $\delta^{18}\text{O}_{\text{NO}_3}$ values we observed are greater than 20‰, thus relatively elevated compared to median values observed by Fripiat et al. (2015). Unsurprisingly, the role of atmospheric NO_3^- was not considered in either Antarctic study (Fripiat et al., 2014, 2015) due to the nitrate-rich surface waters in the Southern Ocean; and no other $\Delta^{17}\text{O}_{\text{NO}_3}$ characterizations exist for sea ice that we are aware of.

The observed $\delta^{15}\text{N}_{\text{NO}_3}$ range in seawater from this study matches observations from the NO_3^- in the Beaufort Sea nutricline ($\sim 7\text{--}8\text{‰}$), however, a broader range is observed for sea ice $\delta^{18}\text{O}_{\text{NO}_3}$ than in the nutricline (0 to 13.8‰ here vs. -1 to 2‰ measured by Granger et al., 2018). This broader range may reflect the environment directly beneath the ice where, at this point in the season, it is possible that melting ice and seawater were mixed. To our knowledge, no $\Delta^{17}\text{O}_{\text{NO}_3}$ measurements have been made on seawater in the region. Finally, there are no other observations of $\delta^{15}\text{N}_{\text{NH}_4}$ or $\delta^{15}\text{N}_{\text{PN}}$ in sea ice or snow from the Arctic region to compare with our results, but the $\delta^{15}\text{N}_{\text{PN}}$ in bottom ice in this study (2.1 to 4.3‰) covers a comparable range to that in spring Antarctic sea ice (1.1 to 4.4‰, Fripiat et al., 2014; $<5\text{‰}$, Fripiat et al., 2015).

The occurrence of $\Delta^{17}\text{O}_{\text{NO}_3}$ indicates that atmospheric NO_3^- is present in the sea ice recovered from the western Arctic basins. Interestingly, $\Delta^{17}\text{O}_{\text{NO}_3} > 0\text{‰}$ is present throughout several of the cores, though this may reflect flushing from the surface (i.e., more advanced desalination) amongst those cores. Lower $\Delta^{17}\text{O}_{\text{NO}_3}$ values than the atmospheric end-member

indicate the co-existence of another NO_3^- source, with $\Delta^{17}\text{O}=0\text{‰}$ (Fig. 3f, S2c). The remaining NO_3^- in sea ice is ostensibly biologically-sourced, produced by nitrification (the oxidation of NH_4^+ and/or NO_2^-) that occurred in sea ice directly (*e.g.*, Fripiat et al., 2014, 2015; Baer et al., 2014, 2015), and/or in seawater before its incorporation into sea ice.

4.1 Quantifying atmospheric versus non-atmospheric nitrate

Simple application of the %AD equation (Section 2.2) based on the observed $\Delta^{17}\text{O}_{\text{NO}_3}$, reveals a range in the contribution of atmospheric NO_3^- to each of the sea ice observations (Fig. 4). In this late-summer Arctic sea ice, all but one of the samples had <40% of (uncycled) atmospheric NO_3^- , while 60 to 100% originated from a non-atmospheric source of NO_3^- (*e.g.*, biological; Fig. 4). Northerly sea ice stations exhibited a lower %AD (<20%, cyan and navy cores) compared to southerly stations (%AD ~30-40%, aqua, beige, salmon cores) with one exception (%AD ~25%, royal blue core) (Fig. 4). For one near-surface sample, we found that 80% of the NO_3^- was atmospheric in origin, and this likely reflects less mixing/cycling in of non-atmospheric NO_3^- consistent with the high percentage of meteoric water based on $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (*i.e.*, cyan and turquoise cores, Fig. 3a).

The multiple isotopic tracers measured here present an opportunity to evaluate hypotheses regarding the dynamics of biological cycling of reactive N in sea ice. Below, we first evaluate whether the range in the %AD is representative of mixing between atmospheric N and seawater-derived nutrients, or significant biological activity within sea ice following incorporation of atmospheric N.

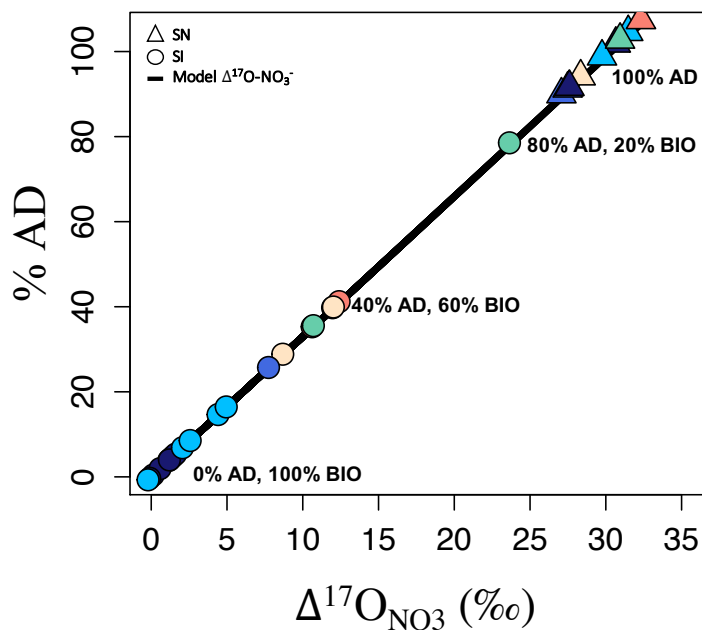


Figure 4: Using modeled $\Delta^{17}\text{O}_{\text{NO}_3}$, the percent atmospheric deposition (%AD) comprised within sea ice is calculated for every observed $\Delta^{17}\text{O}_{\text{NO}_3}$, where snow = 100% AD and the majority of sea ice contains 0-40% AD and 60-100% biological nitrate (BIO). Snow (SN) and sea ice (SI) end-members are represented by triangles and circles colored according to sampling station, while the modeled $\Delta^{17}\text{O}_{\text{NO}_3}$ is in black.

4.2 Sea ice N: a passive reservoir of atmospheric and seawater NO_3^- ?

NO_3^- concentrations were generally more elevated near the surface of the sea ice, decreasing with depth in the ice column to lower values (Fig. 3b). Bulk salinity (Fig. S1b), brine volume fraction (Fig. S1d), and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ data in each of the cores (Fig. 3a) provide evidence that the cores were subjected to recent melting during the summer season, as well as loss and exchange of brines with underlying seawater. Thus, the isotopic composition of NO_3^- and NH_4^+ in sea ice may reflect direct contributions of both dissolved inorganic N from seawater that was incorporated during its formation on the shelf and/or from underlying N-poor seawater during brine exchange, in addition to atmospheric deposition. Indeed, the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ composition for several sea ice samples was intermediate between that of seawater and snow (Fig. 3a).

Given the late summer season ice sampled and brine exchange, we test whether simple end-member mixing of seawater NO_3^- with atmospheric NO_3^- can explain the sea ice N

isotopologue distributions. In Figure 5, we superpose simple end-member mixing curves on the observations (Fig. 5a-c). We use $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ as a conservative tracer (Fig. 5a-c), and also compare the isotopologues of NO_3^- to one another (Fig. 5d-f). Based on the apparent brine loss, the conservative nature of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ is a simplification, however we can use this to test an a priori assumption that we expect a coupling between the $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (*i.e.*, if the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ is reflective of snow, then the $\delta^{18}\text{O}_{\text{NO}_3}$ in snow should reflect atmospheric NO_3^- and vice versa for seawater). The atmospheric NO_3^- end-member is based on the snow observations, because we lack a complete suite of data for the aerosols (*i.e.*, $\Delta^{17}\text{O}_{\text{NO}_3}$) (note that using a mean of the aerosol and snow data for the $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ does not change our conclusions (see Fig. S3)).

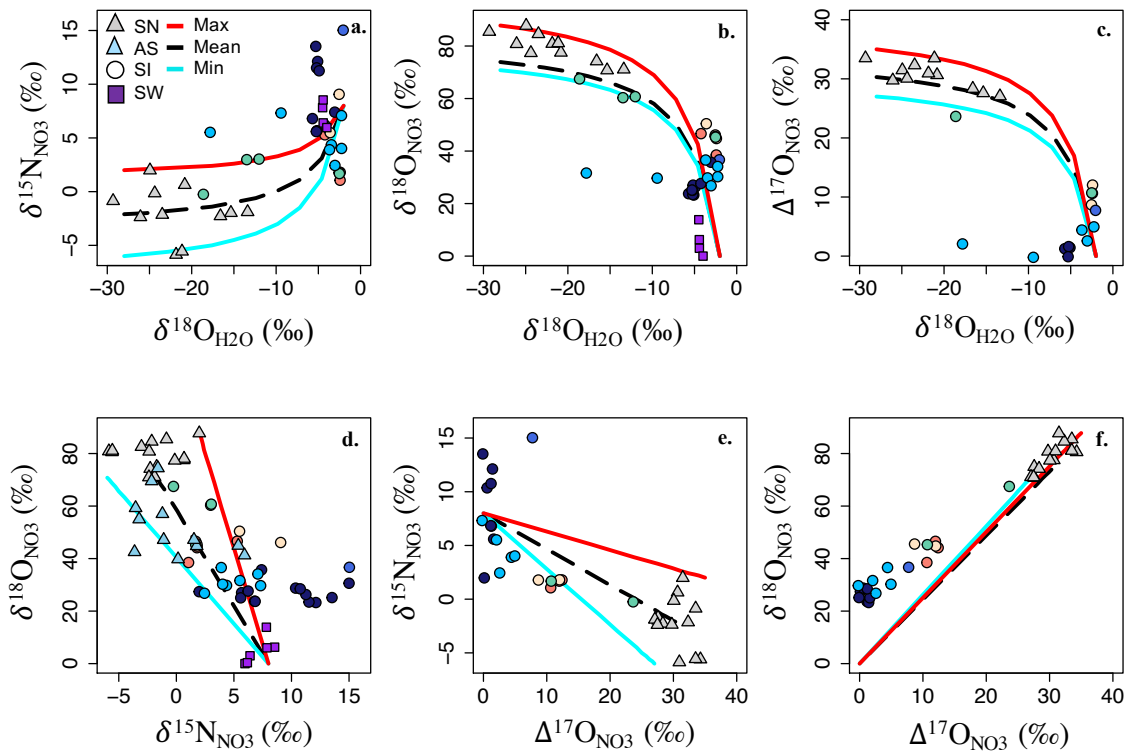


Figure 5: Mixing curves for atmospheric (snow (SN, gray triangles), aerosols (AS, blue triangles)) and seawater (SW, purple squares) end-members. Sea ice (SI, circles) are color-coded by station latitude as noted in Figure 1. The N and O isotopic composition of nitrate are plotted against $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (‰) as follows: a.) $\delta^{15}\text{N}_{\text{NO}_3}$ (‰), b.) $\delta^{18}\text{O}_{\text{NO}_3}$ (‰), and c.) $\Delta^{17}\text{O}_{\text{NO}_3}$ (‰). The nitrate isotopologues are plotted against each other with d.) $\delta^{18}\text{O}_{\text{NO}_3}$ (‰) vs. $\delta^{15}\text{N}_{\text{NO}_3}$ (‰), e.) $\delta^{15}\text{N}_{\text{NO}_3}$ (‰) vs. $\Delta^{17}\text{O}_{\text{NO}_3}$ (‰), and f.) $\delta^{18}\text{O}_{\text{NO}_3}$ (‰) vs. $\Delta^{17}\text{O}_{\text{NO}_3}$ (‰). Curves illustrate mixes with minimum (cyan), mean (black dash) and maximum (red) values for the atmospheric end-member (Table 1).

Mixing curves reveal a potential fit of median $\delta^{15}\text{N}_{\text{NO}_3}$ to $\delta^{18}\text{O}_{\text{H}_2\text{O}}$, but do not encompass the more elevated $\delta^{15}\text{N}_{\text{NO}_3}$ values ($>8\text{‰}$) as well as some lower $\delta^{15}\text{N}_{\text{NO}_3}$ values that correspond with relatively high $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (Fig. 5a). Simple end-member mixing also fails to explain some of the sea ice $\delta^{18}\text{O}_{\text{NO}_3}$, particularly the elevated $\delta^{18}\text{O}_{\text{NO}_3}$ values observed at higher $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (Fig. 5b). As noted earlier, $\delta^{18}\text{O}_{\text{NO}_3}$ values lower than 20‰ were not observed in the sea ice, even at the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values characteristic of seawater. Moreover, $\Delta^{17}\text{O}_{\text{NO}_3} > 0\text{‰}$ were not always correlated with corresponding $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (Fig. 5c) – *i.e.*, a few low $\Delta^{17}\text{O}_{\text{NO}_3}$ values correspond with some of the lowest $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ values that were observed in sea ice, particularly at the northernmost stations (cyan and navy cores). In all, NO_3^- isotope ratios were not adequately captured by conservative end-member mixing relative to corresponding $\delta^{18}\text{O}_{\text{H}_2\text{O}}$.

Even if there is a decoupling of $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and $\delta^{18}\text{O}_{\text{NO}_3}$, which is likely in the event of brine convection and loss, the mixing ratios of NO_3^- isotopologue pools should nevertheless mix conservatively relative to one another. For instance, simple end-member mixing of atmospherically derived NO_3^- with seawater NO_3^- should yield an inverse linear trend between $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$. While some of the observations fit this expectation, coincidently elevated $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ values are not explained, especially at the most northern stations in navy blue (Fig. 5d). Similar to $\delta^{18}\text{O}_{\text{NO}_3}$, conservative mixing should yield an inverse linear relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$, but this fails to explain high $\delta^{15}\text{N}_{\text{NO}_3}$ and low $\Delta^{17}\text{O}_{\text{NO}_3}$ observations (Fig. 5e). Concurrently, sea ice $\delta^{18}\text{O}_{\text{NO}_3}$ values would apparently conform to a linear fit with corresponding $\Delta^{17}\text{O}_{\text{NO}_3}$, but only if the seawater $\delta^{18}\text{O}_{\text{NO}_3}$ end-member were in excess of 20‰ (Fig. 5f), a value that is difficult to justify. Thus, barring highly unusual seawater end-member values for $\delta^{18}\text{O}_{\text{NO}_3}$, simple end-member mixing of atmospheric and seawater NO_3^- does not fully capture the N and O isotopologue distributions that we observed in sea ice NO_3^- .

(Fig. 5d-f).

4.3 Sea ice N: Active in-situ biological cycling?

To further explore mechanisms that might capture the complete distribution for all isotopic tracers, we utilize an isotopic box modeling framework that incorporates biological production and cycling of N within the sea ice (described in Section 2.4.1). We first consider atmospheric NO_3^- and NH_4^+ as deposited on sea ice and cycled biologically therein (“Atm-Only”). We next consider the input of both atmospheric deposition and seawater nutrients, underlying the ice or from the shelf, which are then processed biologically within the sea ice (“Atm + SW” and “Atm + Shelf” scenarios). To compare the model results with observations, we take two approaches. First, we compare across the model scenarios with the range of observations based upon medians and quartiles (box and whisker plots, Fig. S10), considering the range of all model solutions, from initiation to steady-state. Second, we compare the observed isotopologue distributions (property-to-property) to those co-evolved throughout model simulations, from initiation to steady-state (Fig. 6). Here we describe insights gained from the model results, but refer the reader to *SI Text S4* for more detailed descriptions of scenarios tested and the full suite of model results.

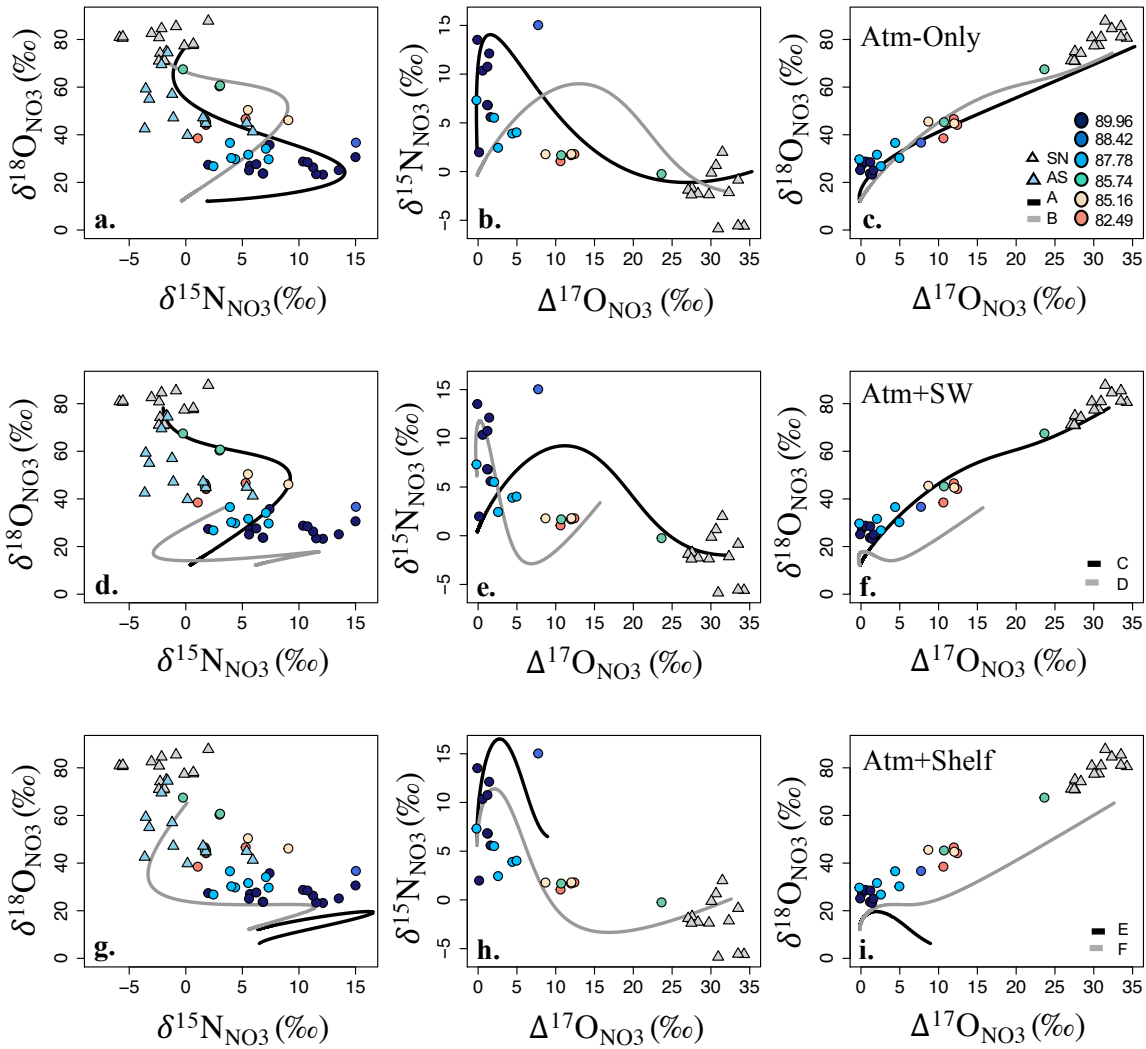


Figure 6: Scenarios Atm-Only (a-c), Atm+SW (d-f), and Atm+Shelf (g-i) illustrate isotopologue comparisons for observations compared to the box model output. Gray triangles represent snow (SN), blue triangles represent aerosols (AS) and sea ice is represented by circles colored by station (SI). The upper and lower bounds tested for all model scenarios are represented with black and grey lines, respectively.

Two representative “Atm-Only” Scenarios, *A* and *B*, were initialized based on the atmospheric observations, with *A* tending to represent the higher end of the parameter ranges (Table S4). For *Scenario B*, the isotope effect for NH_4^+ oxidation was substantially lower than for *A*, as are initial reactant concentrations and isotopologues, and relative biological rates are more conservative (Tables: 2, S3-S4). The production of NO_3^- from the nitrification of atmospheric NH_4^+ should decrease (dilute) the elevated $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ of atmospheric

NO_3^- , whereas partial assimilation of NO_3^- should increase $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ concurrently while leaving the $\Delta^{17}\text{O}_{\text{NO}_3}$ unchanged (because $\delta^{17}\text{O}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ would change mass-dependently).

Sample	$[\text{NO}_3^-]$ (μM)	$\delta^{15}\text{N}_{\text{NO}_3}$ (‰ vs. air)	$\delta^{18}\text{O}_{\text{NO}_3}$ (‰ vs. VSMOW)	$\Delta^{17}\text{O}_{\text{NO}_3}$ (‰ vs. VSMOW)	$[\text{NH}_4^+]$ (μM)	$\delta^{15}\text{N}_{\text{NH}_4}$ (‰ vs. air)
Atm	1.7 – 2.5	-2.0 – 0.0	73.9 – 82.0	30.4	1.0 – 2.5	0.0 – 3.4
SW	0.4 – 1.6	-2.0 – 3.4	35.9 – 77.8	13.8 – 29.9	1.2 – 2.7	4.3 – 6.9
Shelf	1.9–11.7	6.5 – 8.0	0.0 – 10.9	0 – 27.8	4.0–10.0	7.0 – 8.0

Table 2: Ranges of initial end-member values tested in the model scenarios.

The median and range of observed $\delta^{15}\text{N}_{\text{NO}_3}$ are well represented by *Scenario A* (Fig. S10a). While much of the range in $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ are also captured, the model does not reproduce much of the observed elevated O isotopologue distributions (*e.g.*, $\delta^{18}\text{O}_{\text{NO}_3} \sim 35\text{--}45\text{‰}$; $\Delta^{17}\text{O}_{\text{NO}_3} \sim 5\text{--}10\text{‰}$) (Fig. S10b-c). The model also does not capture isotopologue distributions observed for NH_4^+ and PON, yielding much higher $\delta^{15}\text{N}_{\text{NH}_4}$ and much lower $\delta^{15}\text{N}_{\text{PON}}$ compared to the, admittedly few, observations (Fig. S10d-e). Still, the co-evolution of $\delta^{15}\text{N}_{\text{NO}_3}$ vs. $\delta^{18}\text{O}_{\text{NO}_3}$, $\delta^{15}\text{N}_{\text{NO}_3}$ vs. $\Delta^{17}\text{O}_{\text{NO}_3}$, and $\delta^{18}\text{O}_{\text{NO}_3}$ vs. $\Delta^{17}\text{O}_{\text{NO}_3}$ is well described (Fig. 6a-c).

Scenario B, in contrast, while roughly reproducing median observations for $\delta^{15}\text{N}_{\text{NH}_4}$, does not reproduce the observed $\delta^{15}\text{N}_{\text{PON}}$ (Fig. S10), and fails to capture the co-evolution of $\delta^{18}\text{O}_{\text{NO}_3}$ vs. $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ vs. $\Delta^{17}\text{O}_{\text{NO}_3}$ (Fig. 6a-c). Nevertheless, unlike *Scenario A*, this scenario is able to reproduce elevated $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ reflecting the entire observed range in sea ice (Fig. S10b-c). These “Atm-Only” simulations (*A-B*) illustrate that biological processing of atmospheric N in sea ice can explain the observed NO_3^- isotopologue values, but does not explain the observed ranges for $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$.

We next consider the input of both atmospheric deposition and seawater nutrients, which were then processed biologically within the sea ice. Above, in the mixing curves, we considered only the direct physical incorporation of nutrients from the open ocean. While the ice floes were sampled in the open basin, six-month back-trajectories suggest that ice originated from the East Siberian Shelf seas before being advected by the Transpolar Drift to the Makarov Basin and the Beaufort Gyre (Kipp et al., 2018), and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ from this study reveals that the cores consist of varying proportions of seawater (*e.g.*, 18 to 82%). Thus, we tested two different types of boundary conditions: “Atm + SW” (*Scenarios C-D*) and “Atm + Shelf” (*Scenarios E-F*), where the seawater end-member consists of nutrient-deplete seawater from the open basin or nutrient-rich shelf-water, respectively, with corresponding isotopologue end-members. This was done in an attempt to address the changing significance of different nutrient sources along the path of sea ice transport, which hypothetically coincided with seasonal ice evolution (Tables: 2, S3-S4).

The “Atm + SW” model scenarios (*C-D*) tested different proportional mixtures using the mean of atmospheric deposition (*e.g.*, snow) and low-N seawater to initialize the model. Representing upper and lower boundary conditions, *Scenarios C* and *D* reflect 90:10 and 10:90 mixes, respectively (Tables: 2, S3-S4) and we consider a range of 4.3-6.9‰ for $\delta^{15}\text{N}_{\text{NH}_4}$ at model initiation (Tables: 2, S3-S4).

Of the two “Atm +SW” scenarios, only *Scenario C* provides reasonable agreement for all three isotopologues of NO_3^- , although the model again fails to capture $\delta^{15}\text{N}$ of NH_4^+ and PON, as per *Scenarios A* and *B* (Figure S10). In the alternate scenario in which seawater was the primary source of N to sea ice (*Scenario D*), the model greatly underestimates the high $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ (Figure S10), and *only* yields values higher than the observed $\delta^{15}\text{N}_{\text{NO}_3}$ value range. Evidently, a greater initial fraction of atmospheric NO_3^- appears requisite to reproduce the

isotopologue distributions of the NO_3^- remnant in the sea ice sampled. In spite of the poor fit of *Scenario D* to NO_3^- isotopologues, the $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ values simulated therein are within the range of observations, reflecting the sensitivity of the model $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ (as well as $\delta^{15}\text{N}_{\text{NO}_3}$) to higher initial values of $\delta^{15}\text{N}_{\text{NH}_4}$ (see *SI Text S3-S4*; Figs: S5-S10; Table S5 for discussion of model sensitivity and detailed results for scenarios).

In the “Atm + Shelf” scenarios, *Scenario E* was initialized with a 50:50 mixture of shelf N and atmospheric N, while *Scenario F* was initialized with shelf nutrients alone followed by mixing in of atmospheric deposition at the ice surface and continuous biological cycling throughout. For both scenarios, shelf end-member values were based on observations on the Bering and Chukchi shelves (Granger et al., 2011; Brown et al., 2015b; Morales et al., 2014), predicating higher ranges for initial $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NH}_4}$, and their concentrations (Tables: 2, S3-S4).

The “Atm + Shelf” scenarios yielded similarly unsatisfactory results when compared to observed NO_3^- isotopologue values (see *SI Text S4* for details). Nevertheless, as per *Scenario D*, *Scenario F* yields $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ values closer to the observed range, again reflecting the sensitivity of the model output to the higher initial values of $\delta^{15}\text{N}_{\text{NH}_4}$. The disagreement with the NO_3^- isotopologue distributions is evidently greater for the “Atm + Shelf” scenarios than the “Atm + SW” scenarios. This is because the higher initial NO_3^- and NH_4^+ concentrations in shelfwaters (*Scenarios E-F*) tended to swamp the distinctive atmospheric signatures observed in the isotopes.

We use the observed model (property-to-property) behavior to gain insight into the N dynamics that reflect processes in the observed isotopologue ranges. On the basis of the *co-evolution of isotopologues*, *Scenarios A-C* most accurately captured the observed elevated

composition and tight coupling between the O isotopologues (Fig. 6). It is also noteworthy that *Scenarios D-F* illustrated the need for a slightly elevated set of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NH}_4}$ values at model initiation to generate the observed elevated $\delta^{15}\text{N}_{\text{NO}_3}$ ($>0\text{‰}$) with corresponding low $\Delta^{17}\text{O}_{\text{NO}_3}$ ($<5\text{‰}$) (Fig. 6d-i). For *Scenario D*, this is consistent with a higher proportion of seawater NO_3^- , while *Scenarios E-F* reproduced the co-evolution of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ due to more nitrification driven by higher NH_4^+ concentrations from shelfwaters.

In sum, the modeled scenarios that best reproduce the ranges in observations of NO_3^- isotopologue ratios require a significant contribution from atmospheric N and subsequent biological cycling within sea ice. Nevertheless, these scenarios fail in producing observed ranges for $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$, and generated lower median $\delta^{18}\text{O}_{\text{NO}_3}$ values than observed. We therefore revisited the “Atm-Only” model framework (*Scenarios A and B*), which produced the most realistic match with our observations for NO_3^- isotopologues, initializing with a more elevated $\delta^{15}\text{N}_{\text{NH}_4}$ source in an attempt to capture NO_3^- , NH_4^+ and PON observations simultaneously (see *Scenario X*: “High $\delta^{15}\text{N}_{\text{NH}_4}$ Initial” discussed below). *Scenario X* used the same parameter values as in *Scenario B* to test incremental changes in the initial $\delta^{15}\text{N}_{\text{NH}_4}$, and found that the modeled steady-state solutions for $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ were sensitive to this end-member, and changed by 10‰ or more depending on the initial value used (see Table S5, Fig. S5 and S3: *Sensitivity Tests*). Based on these results and to avoid diluting the atmospheric oxygen signatures preserved in the ice, *Scenario X* required relatively low starting concentrations ($\sim 1\text{ }\mu\text{M}$) of an elevated $\delta^{15}\text{N}_{\text{NH}_4}$ (15‰) (Tables: 2, S3-S4) to capture both $\delta^{15}\text{N}_{\text{PON}}$ and $\delta^{15}\text{N}_{\text{NH}_4}$, and still broadly reproduce the observed ranges of $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, and $\Delta^{17}\text{O}_{\text{NO}_3}$ (Fig. S10). However, whether a $\delta^{15}\text{N}_{\text{NH}_4}$ end-member value of 15‰ can be justified is equivocal

based on current constraints in the literature (see further discussion of the feasibility of this parameterization in *SI Text S4*).

Modeling all three isotopologues of NO_3^- allows for quantitatively tracking atmospheric inputs, while also realistically capturing biological consumption and production. The best explanation of the observations rest on an important input of atmospheric nutrients that were biologically processed within the sea ice, albeit with a high starting $\delta^{15}\text{N}_{\text{NH}_4}$. It is also important to consider that a lack of fit between modeled and observed N isotopologues for $\delta^{15}\text{N}_{\text{PON}}$, could be a function of the fact that PON can act as an integrator over time of numerous processes relative to other nutrients “newly” present in the late-summer sea ice sampled in this campaign.

We focus here on trying to best understand the range and relative behavior amongst the observations. The heterogeneous isotopologue patterns observed in sea ice cores could reflect differences in the provenance of NO_3^- and/or biological processing in sea ice at different latitudes. The southerly stations tended towards higher $\Delta^{17}\text{O}_{\text{NO}_3}$, higher $\delta^{18}\text{O}_{\text{NO}_3}$ and lower $\delta^{15}\text{N}_{\text{NO}_3}$ compared to the more northerly stations. These differences could reflect that sea ice at the southerly stations have experienced more flushing. The isotopologue distributions observed in the northern cores more broadly resemble the range and behavior of modeled shelf scenarios (“Atm+Shelf”), while cores collected in the south (particularly the core at station 85.74°N) are more comparable to “Atm-Only” scenario simulations. This is readily apparent in the larger proportion of atmospheric NO_3^- (*i.e.*, higher $\Delta^{17}\text{O}_{\text{NO}_3}$). Stations closest to the pole are suspected to represent first-year ice, while those further south are suspected to reflect multiyear ice (NSIDC, 2015). In young ice, biological processes may be more prominent due to organic material captured in the ice matrix compared to older ice that has experienced seasonal brine flushing (Arrigo et al., 2012). In summary, the heterogeneity observed among the sampled cores

is likely a function of differences in ice age where younger ice is more biologically active and the provenance of nutrients depends on the location of Arctic sea ice formation.

5. Conclusions

Quantifying the presence of atmospheric NO_3^- is a new addition to our understanding of sea ice N cycling. Since $\Delta^{17}\text{O}$ does not change with mass-dependent processes, the low observed $\Delta^{17}\text{O}$ values reflect that an alternative source of NO_3^- must be diluting the atmospheric source in sea ice. Nitrification has been found to be a prominent process occurring in Arctic land-fast (Baer et al., 2014, 2015), Antarctic land-fast and pack ice (Fripiat et al., 2015; Fripiat et al., 2014; Priscu et al., 1990), and it now appears to be significant in open ocean late-summer Arctic pack ice as well. According to Baer et al. (2014), nitrification rates are highest in winter due to light limitation and decreased competition for NH_4^+ between nitrifying microbes and phytoplankton (light-dependent) communities, enabling the oxidation of NH_4^+ to NO_3^- to take place. Light control experiments in shelf bottom and upper halocline waters in the Chukchi and Beaufort Seas also show that above a certain light threshold ($0.11 \text{ mol photons m}^{-2} \text{ day}^{-1}$) nitrification is inhibited (Shiozaki et al., 2019). Importantly, this study suggests that diminishing sea ice cover and enhancing light availability in the surface ocean in a future climate will continue to suppress nitrification in Arctic Ocean environments causing a shift in nitrogen dynamics and ecosystem structure (Shiozaki et al. 2019). If, in fact, nitrification is responsible for the non-atmospheric NO_3^- incorporated in the sea ice, then the importance of this process is possibly dampened in our observations due to differences in sea ice age, suspected seawater-brine exchange and that core collections took place in late-summer.

Our results suggest that: 1) the dilution of atmospheric NO_3^- can be explained by biological production of NO_3^- in sea ice; 2) the fraction of atmospheric NO_3^- in the sea ice samples range from almost negligible to 40%, suggesting that the majority of NO_3^- within sea ice is biologically sourced (60-100%) (Fig. 4); and 3) model scenarios suggest that a suite of isotopologue distributions are consistent with simulations that require a significant input of atmospheric nutrients that are then biologically cycled, and require an initial NH_4^+ source with an expressly elevated $\delta^{15}\text{N}_{\text{NH}_4}$. It is imperative to further refine our understanding of atmospheric sources of $\text{NH}_3/\text{NH}_4^+$ and their respective $\delta^{15}\text{N}$ in an effort to determine the role atmospheric sources play in nitrogen cycling processes in sea ice, and the Arctic Ocean in general. Different sources of N appear to supply in-situ biological processes in Arctic versus Antarctic sea ice, where late-summer Arctic sea ice retains NO_3^- that is atmospheric and biological in origin. While additional constraints are needed to quantify the total contribution to primary production in the surface ocean, we emphasize that the NO_3^- residing in the sea ice cores from this study represents a relatively small, but exogenous nutrient source to oligotrophic regions of the western Arctic Ocean during sea ice evolution/melt. Continued losses of sea ice due to warming ocean temperatures therefore will influence sea surface biogeochemistry in the Arctic Ocean and may result in changes to the distribution of essential nutrients for surface primary production.

6. Acknowledgements

The authors report no conflicts of interest. This work was funded by the National Science Foundation (Grant Number: OCE-1433989 to M. H., OCE-1435002 to J.G. and OCE-1433717 to A.A-I.), the Institute at Brown for Environment and Society (Graduate Research and Travel Grant to S.C.C.), the Brown Graduate School (International Research Travel Grant to S.C.C.) and Brown University's Teaching and Research Awards (to A.M.). Clifton Buck (NSF OCE 1438047), William Landing (NSF OCE 1427266 and NSF-OCE 1355833), and Christopher Marsay provided the atmospheric aerosol samples. We thank the efforts of Phoebe Lam and Yang Xiang for the isotopic analyses of $\delta^{15}\text{N}_{\text{PN}}$ from the cruise, and the following people for their efforts with cruise logistics, sea ice station sampling, and analytical support: David Kadko, Kyle Dilliplaine, Rob Rember, Ruby Ho, Dorothy Fibiger, Joseph Orchard, David Murray, Richard Vachula, the USCGC Healy crew, GEOTRACES community, Andrew Schauer and the UW Isolab. Finally, we thank Dr. Fripiat and an anonymous reviewer for insightful feedback on this manuscript. As part of the GEOTRACES team, data is accessible through the BCO-DMO repository and can be found at: <https://www.bco-dmo.org/project/638812>.

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8. Supplement to: An investigation into the origin of nitrate in Arctic sea ice

Introduction

This supplement includes: four supplemental text sections that describe ancillary sea ice station data compiled from cruise community characterization cores (Text S1), the system of equations used to construct the isotope model (Text S2), sensitivity tests conducted to assess model output sensitivity to initial parameters (Text S3) and discussion of box modeling scenario results (Text S4); nine supplemental figures illustrating sea ice characterization (salinity, temperature, isotopes, concentrations), mixing model results and sensitivity test results; and lastly, four tables describing pooled standard deviations for isotope reference materials, box model parameterizations and results from ammonium isotope sensitivity tests.

Text S1. Ancillary Sea Ice Station Data

Six community cores were sampled from the ice floes at each station to measure core temperature, bulk salinity, brine salinity and brine volume fraction. Brine salinity and brine volume fraction were calculated based on the methods discussed in *Eicken, (2003)*.

Text S2. Box Model of Sea Ice N Dynamics

This section contains the system of equations used to construct the box model, as well as, Tables S3-S4 with values used to initiate each scenario (A-X) and steady-state evolution of isotopologues in each scenario tested (Fig. S4).

Nitrate

1. $\alpha = \frac{\text{light } k}{\text{heavy } k} = (\epsilon/1000) + 1$
2. $^{15}R_{\text{NO}_3} = ((\delta^{15}\text{N}_{\text{NO}_3}/1000) + 1) * 0.00367$
3. $^{15}F_{\text{NO}_3} = \frac{^{15}\text{N}_{\text{NO}_3}}{(^{15}\text{N}_{\text{NO}_3} + ^{14}\text{N}_{\text{NO}_3})}$
4. $^{18}R_{\text{NO}_3} = ((\delta^{18}\text{O}_{\text{NO}_3}/1000) + 1) * 0.002005$
5. $^{18}F_{\text{NO}_3} = \frac{^{18}\text{O}_{\text{NO}_3}}{(^{18}\text{O}_{\text{NO}_3} + ^{16}\text{O}_{\text{NO}_3})}$

6. $^{17}R_{NO_3} = ((\delta^{17}O_{NO_3}/1000)+1)*0.00038$
7. $^{17}F_{NO_3} = ^{17}O_{NO_3}/(^{17}O_{NO_3} + ^{16}O_{NO_3})$
8. $\delta^{15}N_{NO_3} = (((^{15}N_{NO_3}/^{14}N_{NO_3})/0.00367)-1)*1000$
9. $\delta^{18}O_{NO_3} = (((^{18}O_{NO_3}/^{16}O_{NO_3})/0.002005)-1)*1000$
10. $\delta^{17}O_{NO_3} = (((^{17}O_{NO_3}/^{16}O_{NO_3})/0.00038)-1)*1000$
11. $\Delta^{17}O_{NO_3} = \delta^{17}O_{NO_3} - 0.52 * \delta^{18}O_{NO_3}$
12. $\% \Delta^{17}O_{NO_3} = \Delta^{17}O_{NO_3} / \Delta^{17}O_{NO_3 \text{ atm}} * 100$
13. $\delta^{15}N_{NO_3\text{-prod}} = (((^{15}N_{NH_4\text{-oxid}}/^{14}N_{NH_4\text{-oxid}})/0.00367)-1)*1000$
14. $\delta^{18}O_{NO_3\text{-prod}} = (((^{18}O_{NO_3\text{-prod}}/^{16}O_{NO_3\text{-prod}})/0.002005)-1)*1000$
15. $[^{18}O_{NO_3}]_{(t1)} = NO_3^- * ^{18}F_{NO_3\text{-atm}}$
16. $[^{18}O_{NO_3}]_{(t+1)} = ^{18}O_{NO_3(t1)} - ^{18}O_{NO_3\text{-uptake}(t1)} + ^{18}O_{NO_3\text{-prod}(t1)}$
17. $[^{17}O_{NO_3}]_{(t1)} = NO_3^- * ^{17}F_{NO_3\text{-atm}}$
18. $[^{17}O_{NO_3}]_{(t+1)} = ^{17}O_{NO_3(t1)} - ^{17}O_{NO_3\text{-uptake}(t1)} + ^{17}O_{NO_3\text{-prod}(t1)}$
19. $[^{16}O_{NO_3}]_{(t1)} = NO_3^- * (1 - ^{18}F_{NO_3\text{-atm}} - ^{17}F_{NO_3\text{-atm}})$
20. $[^{16}O_{NO_3}]_{(t+1)} = ^{16}O_{NO_3(t1)} - ^{16}O_{NO_3\text{-uptake}(t1)} + ^{16}O_{NO_3\text{-prod}(t1)}$
21. $[^{15}N_{NO_3}]_{(t1)} = NO_3^- * ^{15}F_{NO_3\text{-atm}}$
22. $[^{15}N_{NO_3}]_{(t+1)} = ^{15}N_{NO_3(t1)} - ^{15}N_{NO_3\text{-uptake}(t1)} + ^{15}N_{NH_4\text{-oxid}(t1)}$
23. $[^{14}N_{NO_3}]_{(t1)} = NO_3^- * (1 - ^{15}F_{NO_3\text{-atm}})$
24. $[^{14}N_{NO_3}]_{(t+1)} = ^{14}N_{NO_3(t1)} - ^{14}N_{NO_3\text{-uptake}(t1)} + ^{14}N_{NH_4\text{-oxid}(t1)}$
25. $[^{15}N_{NO_3\text{-uptake}}]_{(t+1)} = dt * \text{uptake-rate}_{NO_3} * NO_3^-(t) * ^{15}F_{NO_3(t)} / \alpha^{15}_{NO_3\text{-uptake}}$
26. $[^{14}N_{NO_3\text{-uptake}}]_{(t+1)} = dt * \text{uptake-rate}_{NO_3} * NO_3^-(t) * (1 - ^{15}F_{NO_3(t)})$
27. $[^{18}O_{NO_3\text{-uptake}}]_{(t+1)} = dt * \text{uptake-rate}_{NO_3} * NO_3^-(t) * ^{18}F_{NO_3(t)} / \alpha^{18}_{NO_3\text{-uptake}}$
28. $[^{17}O_{NO_3\text{-uptake}}]_{(t+1)} = dt * \text{uptake-rate}_{NO_3} * NO_3^-(t) * ^{17}F_{NO_3(t)} / \alpha^{17}_{NO_3\text{-uptake}}$
29. $[^{16}O_{NO_3\text{-uptake}}]_{(t+1)} = dt * \text{uptake-rate}_{NO_3} * NO_3^-(t) * (1 - ^{18}F_{NO_3(t)} - ^{17}F_{NO_3(t)})$
30. $[^{18}O_{NO_3\text{-prod}}]_{(t+1)} = dt * \text{oxid-rate}_{NH_4} * NH_4^+(t) * ((2/3) * ^{18}F_{H_2O} * \alpha^{18}_{NO_2\text{-H}_2\text{O-equil}} + (1/3) * ^{18}F_{H_2O} / \alpha^{18}_{NO_2\text{-H}_2\text{O-NXR}})$
31. $[^{17}O_{NO_3\text{-prod}}]_{(t+1)} = dt * \text{oxid-rate}_{NH_4} * NH_4^+(t) * (((2/3) * ^{17}F_{H_2O} * ((\alpha^{18}_{NO_2\text{-H}_2\text{O-equil}} - 1) * 0.5 + 1)) + ((1/3) * ^{17}F_{H_2O} / ((\alpha^{18}_{NO_2\text{-H}_2\text{O-NXR}} - 1) * 0.5 + 1))))$
32. $[^{16}O_{NO_3\text{-prod}}]_{(t+1)} = dt * \text{oxid-rate}_{NH_4} * NH_4^+(t) * ((2/3) * (1 - ^{18}F_{H_2O} - ^{17}F_{H_2O}) + (1/3) * (1 - ^{18}F_{H_2O} - ^{17}F_{H_2O}))$
33. $^{17}\alpha_{NO_3 \text{ uptake}} = (^{15}\alpha_{NO_3 \text{ uptake}} - 1) * 0.52 + 1$

Particulate Organic Nitrogen

34. $[PON]_{(t1)} = ^{15}N_{PON} + ^{14}N_{PON}$
35. $[PON]_{(t+1)} = PON_{(t)} + ^{15}N_{NO_3\text{-uptake}(t)} + ^{14}N_{NO_3\text{-uptake}(t)} + ^{15}N_{NH_4\text{-uptake}(t)} + ^{14}N_{NH_4\text{-uptake}(t)} - ^{15}N_{NH_4\text{-prod}(t)} - ^{14}N_{NH_4\text{-prod}(t)}$
36. $[^{15}N_{PON}]_{(t+1)} = ^{15}N_{PON(t)} + ^{15}N_{NO_3\text{-uptake}(t)} + ^{15}N_{NH_4\text{-uptake}(t)} - ^{15}N_{NH_4\text{-prod}(t)}$
37. $[^{14}N_{PON}]_{(t+1)} = ^{14}N_{PON(t)} + ^{14}N_{NO_3\text{-uptake}(t)} + ^{14}N_{NH_4\text{-uptake}(t)} - ^{14}N_{NH_4\text{-prod}(t)}$
38. $^{15}F_{PON} = ^{15}N_{PON} / (^{15}N_{PON} + ^{14}N_{PON})$
39. $\delta^{15}N_{PON} = (((^{15}N_{PON}/^{14}N_{PON})/0.00367)-1)*1000$

Ammonium

40. $[NH_4^+]_{(t+1)} = ^{15}N_{NH_4(t)} + ^{14}N_{NH_4(t)}$
41. $\delta^{15}N_{NH_4} = (((^{15}N_{NH_4}/^{14}N_{NH_4})/0.00367)-1)*1000$
42. $^{15}R_{NH_4} = ((\delta^{15}N_{NH_4}/1000)+1)*0.00367$

43. $^{15}\text{F}_{\text{NH}_4} = ^{15}\text{N}_{\text{NH}_4} / (^{15}\text{N}_{\text{NH}_4} + ^{14}\text{N}_{\text{NH}_4})$
44. $[^{15}\text{N}_{\text{NH}_4}]_{(t+1)} = ^{15}\text{N}_{\text{NH}_4(t)} - ^{15}\text{N}_{\text{NH}_4\text{-oxid}} + ^{15}\text{N}_{\text{NH}_4\text{-prod}} - ^{15}\text{N}_{\text{NH}_4\text{-uptake}(t)}$
45. $[^{15}\text{N}_{\text{NH}_4}]_{(t)} = \text{NH}_4^+ * ^{15}\text{F}_{\text{NH}_4}$
46. $[^{14}\text{N}_{\text{NH}_4}]_{(t)} = \text{NH}_4^+ * (1 - ^{15}\text{F}_{\text{NH}_4})$
47. $[^{14}\text{N}_{\text{NH}_4}]_{(t+1)} = ^{14}\text{N}_{\text{NH}_4(t)} - ^{14}\text{N}_{\text{NH}_4\text{-oxid}} + ^{14}\text{N}_{\text{NH}_4\text{-prod}} - ^{14}\text{N}_{\text{NH}_4\text{-uptake}(t)}$
48. $[^{15}\text{N}_{\text{NH}_4\text{-prod}}]_{(t+1)} = \text{PON}_{(t)} * dt * \text{prod-rate}_{\text{NH}_4} * ^{15}\text{F}_{\text{PON}(t)}$
49. $[^{14}\text{N}_{\text{NH}_4\text{-prod}}]_{(t+1)} = \text{PON}_{(t)} * dt * \text{prod-rate}_{\text{NH}_4} * (1 - ^{15}\text{F}_{\text{PON}(t)})$
50. $[^{15}\text{N}_{\text{NH}_4\text{-oxid}}]_{(t+1)} = dt * \text{oxid-rate}_{\text{NH}_4} * \text{NH}_4^+ * ^{15}\text{F}_{\text{NH}_4(t)} / \alpha^{15}_{\text{NH}_4\text{-oxid}}$
51. $[^{14}\text{N}_{\text{NH}_4\text{-oxid}}]_{(t+1)} = dt * \text{oxid-rate}_{\text{NH}_4} * \text{NH}_4^+ * (1 - ^{15}\text{F}_{\text{NH}_4(t)})$
52. $[^{15}\text{N}_{\text{NH}_4\text{-uptake}}]_{(t+1)} = dt * \text{assim-rate}_{\text{NH}_4} * \text{NH}_4^+ * ^{15}\text{F}_{\text{NH}_4(t)} / \alpha^{15}_{\text{NH}_4\text{-uptake}}$
53. $[^{14}\text{N}_{\text{NH}_4\text{-uptake}}]_{(t+1)} = dt * \text{assim-rate}_{\text{NH}_4} * \text{NH}_4^+ * (1 - ^{15}\text{F}_{\text{NH}_4(t)})$

Water

54. $^{18}\text{R}_{\text{H}_2\text{O}} = (\delta^{18}\text{O}_{\text{H}_2\text{O}}/1000) + 1) * 0.002005$
55. $^{18}\text{F}_{\text{H}_2\text{O}} = ^{18}\text{R}_{\text{H}_2\text{O}} / (^{18}\text{R}_{\text{H}_2\text{O}} + 1)$
56. $\delta^{17}\text{O}_{\text{H}_2\text{O}} = \delta^{18}\text{O}_{\text{H}_2\text{O}} * 0.52$
57. $^{17}\text{R}_{\text{H}_2\text{O}} = (\delta^{17}\text{O}_{\text{H}_2\text{O}}/1000) + 1) * 0.00038$
58. $^{17}\text{F}_{\text{H}_2\text{O}} = ^{17}\text{R}_{\text{H}_2\text{O}} / (^{17}\text{R}_{\text{H}_2\text{O}} + 1)$
59. $^{18}\text{R}_{\text{NO}_2\text{-H}_2\text{O-EQUIL}} = ((\delta^{18}\text{O}_{\text{H}_2\text{O}} + 13)/1000 + 1) * 0.002005$
60. $^{18}\text{F}_{\text{NO}_2\text{-H}_2\text{O-EQUIL}} = ^{18}\text{R}_{\text{NO}_2\text{-H}_2\text{O-EQUIL}} / (^{18}\text{R}_{\text{NO}_2\text{-H}_2\text{O-EQUIL}} + 1)$

Text S3. Sensitivity Tests

Several parameters were tested to assess model sensitivity. The model output was considered insensitive, moderately sensitive, or sensitive to parameter changes if isotopic yields changed by: 0-5‰, 5-10‰, or >10‰, respectively. Initial $\delta^{15}\text{N}_{\text{NO}_3}$ values between -10 to -2‰ and $\delta^{15}\text{N}_{\text{NH}_4}$ values between -22 to 2‰ were tested to assess the response of $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ output (Fig. S5). Isotope effects were varied according to published ranges including: $\alpha^{15}\text{N}$ and $\alpha^{18}\text{O}$ of NO_3^- uptake (between 1.005 to 1.010, Difiore et al. 2009), $\alpha^{15}\text{N}$ of NH_4^+ oxidation (1.014, 1.019 and 1.030, Casciotti et al. 2003) and $\alpha^{15}\text{N}$ of NH_4^+ uptake (1.000 to 1.020‰, Liu et al., 2013; Pennock et al., 1996; Hoch et al., 1992; Fig. S6). Nine different initial concentrations of NO_3^- and NH_4^+ (e.g. 1 to 9 $\mu\text{mol L}^{-1}$) were tested to see how these changes alter the steady-state solutions for N and O isotope ratios of all N pools (Fig. S7-S8). Finally, the sensitivity of isotopologue ratios to biological rates were also tested and compared to isotopic

property plots of the observations. A given rate was tested at three different values, while the isotope effects, concentrations, isotope compositions and other rates were kept constant (or as described in Tables: S3-S4). Rates for NO_3^- uptake, NH_4^+ oxidation and NH_4^+ assimilation were tested at 0.2, 0.4, and 0.8 days^{-1} , while NH_4^+ production was tested at 0.02, 0.04 and 0.08 days^{-1} , which were selected to represent a minimum, median and maximum value (Fig. S9).

Model sensitivity tests reveal that of all parameters tested, initialization of NH_4^+ isotopic composition ($\delta^{15}\text{N}_{\text{NH}_4}$) result in the most drastic changes in model output (Table S5; Fig. S5b). Modeled output for $\delta^{15}\text{N}_{\text{NH}_4}$, $\delta^{15}\text{N}_{\text{NO}_3}$, and $\delta^{15}\text{N}_{\text{PON}}$ are insensitive to initial $\delta^{15}\text{N}_{\text{NO}_3}$, but sensitive to initial $\delta^{15}\text{N}_{\text{NH}_4}$ (change in output on the order of 15‰; Fig. S5a-b). $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ are considered insensitive to initial $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ (Fig. S5c). Of the isotope effects tested, $\delta^{15}\text{N}_{\text{NH}_4}$, and $\delta^{15}\text{N}_{\text{PON}}$ are insensitive to the isotope effects for NH_4^+ oxidation and $^{15}\text{N}\text{-NO}_3^-$ uptake, while $\delta^{15}\text{N}_{\text{NO}_3}$ is moderately sensitive to these initial values (Fig. S6a, c). $\Delta^{17}\text{O}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ are deemed insensitive to changes in $^{18}\text{O}\text{-NO}_3^-$ uptake (Fig. S6b). $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ are sensitive to initial values used for NH_4^+ uptake, while $\delta^{15}\text{N}_{\text{PON}}$ is insensitive (Fig. S6d). Finally, initial concentrations of NH_4^+ and NO_3^- (e.g., using a range of values) yield minimal effect on isotopic output (e.g. less than 5‰ change for $\delta^{15}\text{N}_{\text{NH}_4}$, $\delta^{15}\text{N}_{\text{NO}_3}$, and $\delta^{15}\text{N}_{\text{PON}}$; no change for $\delta^{18}\text{O}_{\text{NO}_3}$ or $\Delta^{17}\text{O}_{\text{NO}_3}$), thus are deemed insensitive (Fig. S7-S8).

Sensitivity tests incorporating biological rate changes, suggest model output for the isotopes of NO_3^- are least sensitive to NH_4^+ oxidation (Fig. S9d-f), while $\delta^{15}\text{N}_{\text{NO}_3}$ is most sensitive to NO_3^- uptake and NH_4^+ production/assimilation (Fig. S9a-c, g-l), with NH_4^+ assimilation adjustments doing the best job of potentially fitting the behavior of the observations for $\delta^{15}\text{N}_{\text{NO}_3}$ vs $\delta^{18}\text{O}_{\text{NO}_3}$ comparisons (black line represents *Scenario A*; Fig S9j-l) followed by changes to NO_3^- uptake which achieves elevated $\delta^{15}\text{N}_{\text{NO}_3}$ values close to 15‰ observed within

the sea ice. Oxygen isotopes are most effected by NH_4^+ assimilation, which is best illustrated by Fig S9j-l. Upon this determination, the ratio of rates were adjusted until reaching the prescribed best model fit, which involved setting the NO_3^- uptake and NH_4^+ oxidation rates at 0.6 and 0.3 days^{-1} , while NH_4^+ production and assimilation were lowered to 0.03 and 0.08 days^{-1} (Table 2, S3-S4). Contrary to the sensitivities observed from individual rate changes in Figure S9, the challenge involved in modulating rates while fitting the isotopic-property plots for the observations involves the interaction between multiple parameters at once and therefore might require fine-tuning parameters that do not initially appear to drive the model output.

Text S4. Isotope Box Modeling Results

The “Atm + SW” *Scenario C* produces similar results to the “Atm-Only” *Scenario B*, owing to the fact that it is initialized with a majority of atmospheric N. This model scenario captures the ranges in the observations for NO_3^- isotopologues, but fails to reproduce isotopic observations for NH_4^+ and PON. Unlike *Scenario B*, however, upper bounds of the $\delta^{15}\text{N}_{\text{NO}_3}$ range are not fully captured (Fig. S10a). Given a greater initial proportion of seawater nutrients (*Scenario D*), observed ranges for $\delta^{18}\text{O}_{\text{NO}_3}$ (and $\Delta^{17}\text{O}_{\text{NO}_3}$) are not captured by the model (Fig. S10b-c), and fails to reproduce the entire range of elevated $\delta^{15}\text{N}_{\text{NO}_3}$ (e.g., >11-12‰; Fig. S10a). However, this scenario better captures the $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ (Fig. S10d-e). The apparent “fit” to $\delta^{15}\text{N}_{\text{NH}_4}$ and $\delta^{15}\text{N}_{\text{PON}}$ values derives largely from the higher starting values of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NH}_4}$ prescribed for the seawater-dominant mixture (10:90). In all, the above scenarios provide a less satisfactory explanation for the observed sea ice isotopologue distributions than the “Atm-Only” scenarios (Fig. S10, 6d-f), largely because the incorporation and cycling of low concentration, open-basin NO_3^- and NH_4^+ leads to lower than observed oxygen isotopologues ratios.

In *Scenario E*, the initial mixing of shelf nutrients with atmospherically derived nutrients reduces the distinctive atmospheric signatures in sea ice at the onset of the simulation (Fig. S10; see also the temporal evolution of model result in Fig. S4). As such, this scenario leads to disagreement with the observed $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$, as well as the lower end of $\delta^{15}\text{N}_{\text{NO}_3}$ (Fig. S10-c). On the other hand, this scenario does capture the upper range of elevated $\delta^{15}\text{N}_{\text{NO}_3}$ values, as well as the positive $\delta^{15}\text{N}_{\text{PON}}$, but produces $\delta^{15}\text{N}_{\text{NH}_4}$ that is too high (Fig. S10a, d-e).

Scenario F implicitly models the biological processing of shelf-sourced nutrients first followed by the incorporation and processing of an atmospherically sourced N pool. This reflects that atmospheric deposition is stored near the sea ice surface and furthest from biological activity near the sea ice base (Horner, 1985). Over time, we speculate that atmospheric nutrients become more accessible for biological consumption and production, possibly as a result of drainage networks during desalination that may aid in transport from surface ice to biological communities located near the pack ice base. This scenario yields some of the highest $\delta^{15}\text{N}_{\text{PON}}$ values, but much narrower ranges in the NO_3^- isotopologues than observed (Fig. S10a,e). Moreover, *Scenario F* greatly underestimates the $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ and overestimates $\delta^{15}\text{N}_{\text{NH}_4}$ (Fig. S10b-d). While *F* captures the general patterns in behavior observed between $\delta^{15}\text{N}_{\text{NO}_3}$ and both oxygen isotopologues, it greatly underestimates the $\delta^{18}\text{O}_{\text{NO}_3}$ such that the linear relationship between $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ is underestimated, and the high $\delta^{15}\text{N}_{\text{NO}_3}$ associated with low values for $\delta^{18}\text{O}_{\text{NO}_3}$ are also not captured (Fig. 6g-i).

Scenario X comes the closest to capturing both the $\delta^{15}\text{N}_{\text{PON}}$ and $\delta^{15}\text{N}_{\text{NH}_4}$, and broadly reproducing the observed ranges of $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, and $\Delta^{17}\text{O}_{\text{NO}_3}$, especially in comparison to the “Atm+SW” and “Atm+Shelf” scenarios (Fig. S10). However, whether a $\delta^{15}\text{N}_{\text{NH}_4}$ end-member value of 15‰ can be justified is equivocal. Values this high have not been previously

observed in atmospheric samples from the Arctic, although existing data are limited. $\delta^{15}\text{N}_{\text{NH}_4}$ values as high as 15‰ have been observed in rainwater in the subtropics (Altieri et al., 2014). On the other hand, elevated $\delta^{15}\text{N}_{\text{NH}_4}$ values could originate from the shelf. At the onset of ice formation in shelf polynyas, NH_4^+ could be the dominant inorganic N species due to recycling of phytoplankton blooms, as observed on the Bering Sea shelf (Whitledge et al., 1986). In turn, relatively elevated $\delta^{15}\text{N}_{\text{NH}_4}$ in shelf waters are observed, due, in part, to benthic processing (Granger et al., 2011; Morales et al. 2014; Brown et al., 2015b). In this case, however, an elevated concentration and isotopic composition of NH_4^+ must be incorporated in the sea ice with little to no seawater NO_3^- , which while not impossible could be deemed overly specific as a constraint. Moreover, the relative concentration of NH_4^+ would need to remain low to minimize dilution of the oxygen isotopologue signatures. Thus, the isotopologue distributions in the ice column are only reproduced given a specifically elevated $\delta^{15}\text{N}_{\text{NH}_4}$ end-member composition, the potential origins of which are debatable. Additional sources of elevated $\delta^{15}\text{N}_{\text{NH}_4}$ to consider in future study could be: NH_4^+ originating from bird guano emissions and/or wildfires (Wentworth et al., 2016; Blackall et al., 2007); and microbial sea ice communities experiencing osmotic stress that release NH_4^+ during stark changes in salinity (Firth et al., 2016), which are likely to become more common as the surface ocean warms.

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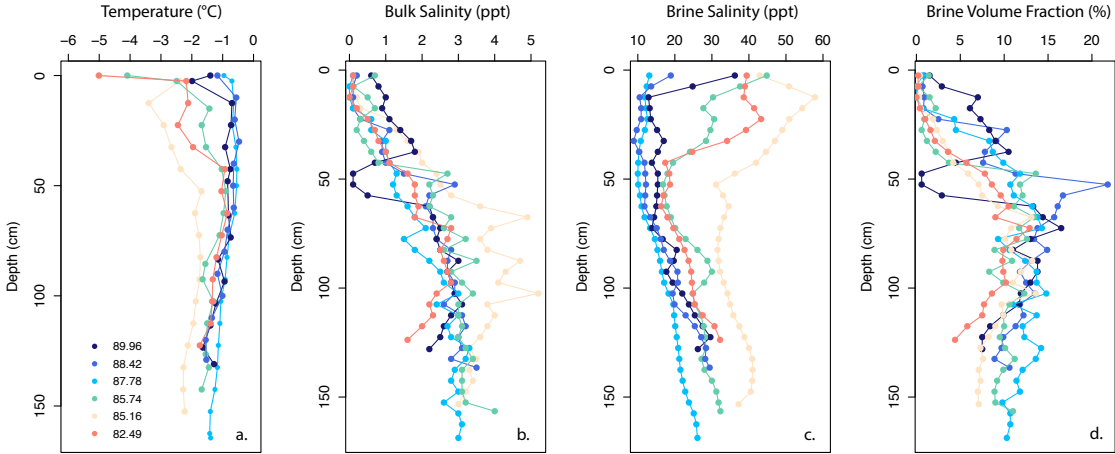


Figure S1. Ancillary data from each sea ice station including: a.) temperature (°C), b.) bulk salinity (ppt), c.) brine salinity (ppt), and d.) brine volume fraction (%). All ancillary data provided by A. Aguilar-Islas.

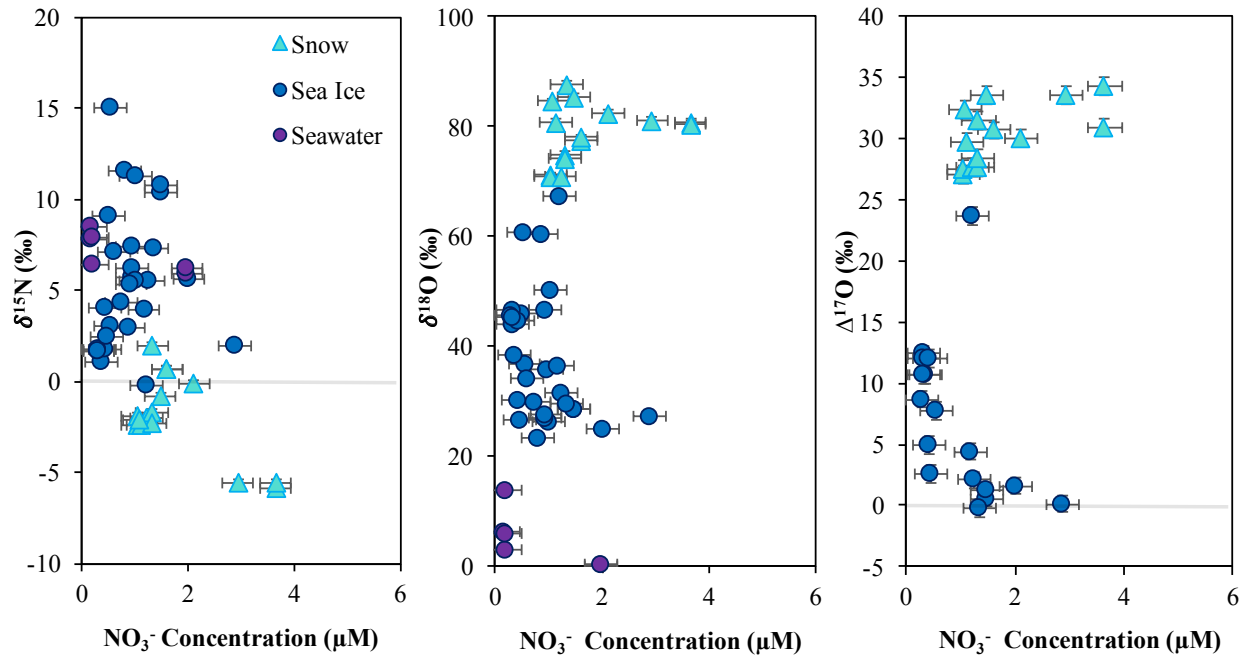


Figure S2. Stable isotopes of nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, and $\Delta^{17}\text{O}_{\text{NO}_3}$) versus nitrate concentrations (μM). Error bars $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ are ± 0.2 , ± 0.7 and ± 0.9 ‰, respectively. Error bars for the $\Delta^{17}\text{O}$ of seawater are ± 1.7 ‰ and based on the standard deviation of 2 replicate measurements using the isotope dilution method. Nitrate concentrations are ± 0.3 μM . Gray lines demarcate the 0 ‰ line.

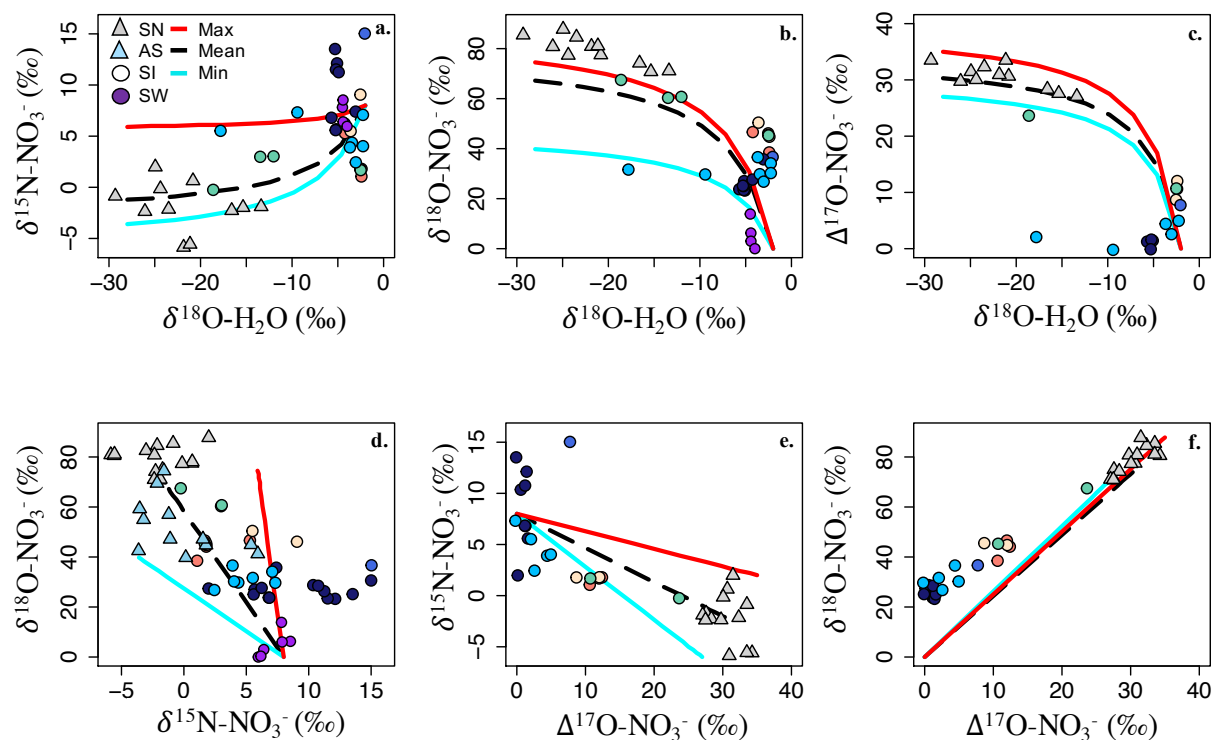


Figure S3. Similar to Figure 5, mixing curves for atmospheric (SN, gray triangles), aerosols (AS, blue triangles) and seawater (SW, purple circles) end-members. Sea ice (SI, circles) are color-coded by station latitude as noted in Figure 1. The N and O isotopic composition of nitrate are plotted against $\delta^{18}\text{O-H}_2\text{O}$ (‰) as follows: a.) $\delta^{15}\text{N-NO}_3^-$ (‰), b.) $\delta^{18}\text{O-NO}_3^-$ (‰), and c.) $\Delta^{17}\text{O-NO}_3^-$ (‰). The nitrate isotopologues are plotted against each other with d.) $\delta^{18}\text{O-NO}_3^-$ (‰) vs. $\delta^{15}\text{N-NO}_3^-$ (‰), e.) $\delta^{15}\text{N-NO}_3^-$ (‰) vs. $\Delta^{17}\text{O-NO}_3^-$ (‰), and f.) $\delta^{18}\text{O-NO}_3^-$ (‰) vs. $\Delta^{17}\text{O-NO}_3^-$ (‰). Curves illustrate mixes with mean (black dash), minimum (cyan), and maximum (red) values for the atmospheric end-member. TotAD represents a mix of aerosols and snow, where mixing curve values include: TotAD Mean (-1.2‰, 67.2‰, and 30.3‰ for $\delta^{15}\text{N-NO}_3^-$, $\delta^{18}\text{O-NO}_3^-$ and $\Delta^{17}\text{O-NO}_3^-$, respectively), Min (-3.6‰, 39.8‰, and 29.0‰, respectively), Max (5.9‰, 74.5‰, and 33.5‰, respectively), and the nitrate concentration was held at 1.3 μM . $\delta^{18}\text{O-H}_2\text{O}$ is represented by snow value (-28‰) for all mixing curves.

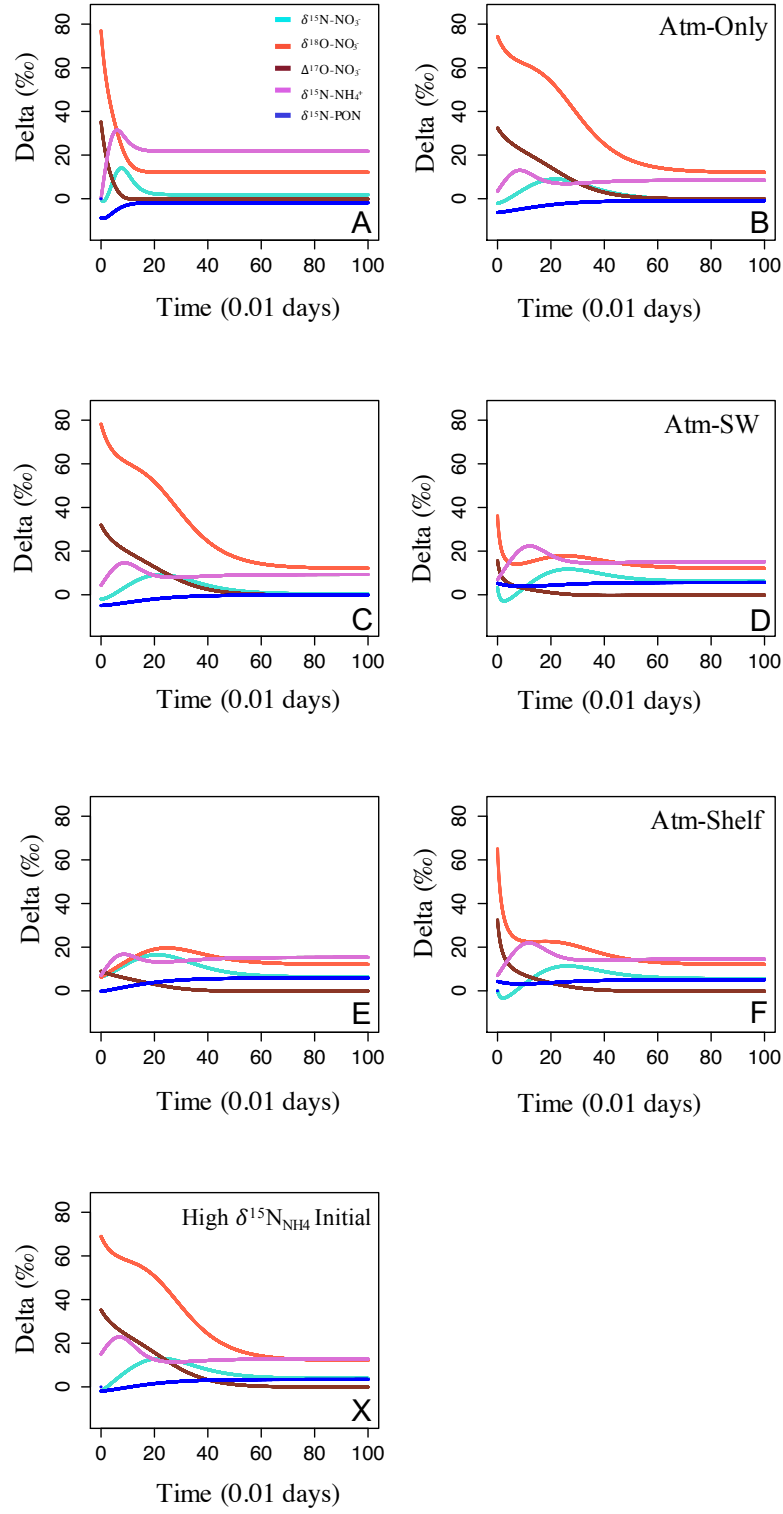


Figure S4. Isotopologue composition for “Atm-Only” (A-B), “Atm+SW” (C-D), “Atm+Shelf” (E-F), and High $\delta^{15}\text{N}_{\text{NH}_4}$ Initial (X) model scenarios tested from initiation to steady-state.

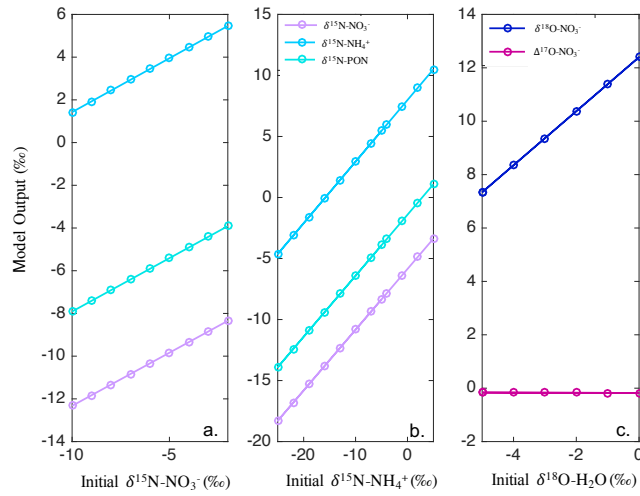


Figure S5. Sensitivity of initial isotopologue composition ($\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{15}\text{N}_{\text{NH}_4}$, $\delta^{18}\text{O}_{\text{H}_2\text{O}}$) on N and O isotopologue steady-state solutions ($\delta^{15}\text{N}_{\text{NO}_3}$ (purple), $\delta^{15}\text{N}_{\text{NH}_4}$ (blue), $\delta^{15}\text{N}_{\text{PON}}$ (green), $\delta^{18}\text{O}_{\text{NO}_3}$ (dark blue), $\Delta^{17}\text{O}_{\text{NO}_3}$ (magenta)). N isotopologues are mildly sensitive to initial nitrate composition as the output changes by 4‰, but not as sensitive as it is to starting ammonium composition, which changes by as much as 15‰. While $\Delta^{17}\text{O}_{\text{NO}_3}$ is insensitive to the isotopic composition of water, the $\delta^{18}\text{O}_{\text{NO}_3}$ is quite sensitive as it shifts from 7 to 12‰ as the starting composition moves from -5 to 0‰.

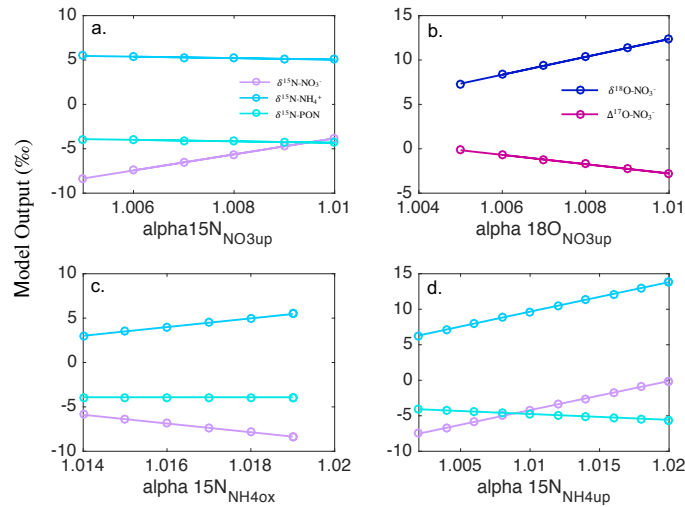


Figure S6. Sensitivity of isotope effects on modeled isotopologue steady-state solutions of nitrogen and oxygen including: a.) nitrate uptake for N isotopologues (output = $\delta^{15}\text{N}_{\text{NO}_3}$ (purple), $\delta^{15}\text{N}_{\text{NH}_4}$ (blue), $\delta^{15}\text{N}_{\text{PON}}$ (green)), b.) nitrate uptake for oxygen isotopologues ($\delta^{18}\text{O}_{\text{NO}_3}$ = dark blue, $\Delta^{17}\text{O}_{\text{NO}_3}$ = magenta), c.) ammonium oxidation, and d.) ammonium uptake for nitrogen isotopologues.

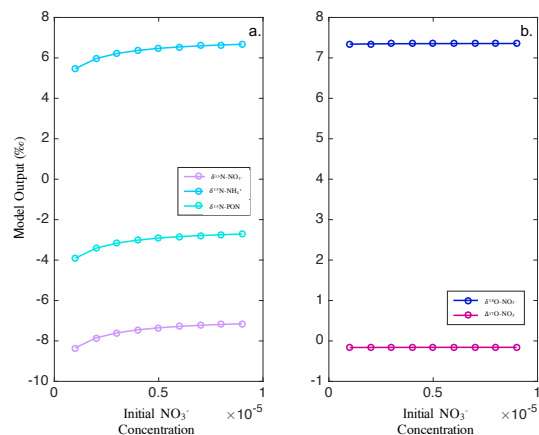


Figure S7. Sensitivity of initial nitrate concentrations on modeled steady-state solutions for all five isotopologues ($\delta^{15}\text{N}_{\text{NO}_3}$ (purple), $\delta^{15}\text{N}_{\text{NH}_4}$ (blue), $\delta^{15}\text{N}_{\text{PON}}$ (green), $\delta^{18}\text{O}_{\text{NO}_3}$ (dark blue), $\Delta^{17}\text{O}_{\text{NO}_3}$ (magenta)). Concentrations tested range from 1 – 9 $\mu\text{moles L}^{-1}$. Oxygen isotopes appear insensitive to nitrate concentrations, while N isotopologues are mildly sensitive (<1‰ for concentration range tested).

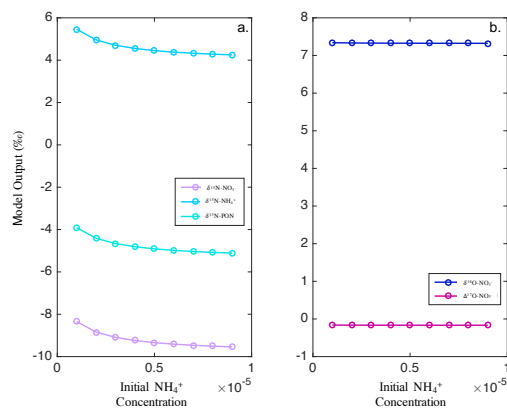


Figure S8. Sensitivity of initial ammonium concentrations on modeled steady-state solutions for all five isotopologues ($\delta^{15}\text{N}_{\text{NO}_3}$ (purple), $\delta^{15}\text{N}_{\text{NH}_4}$ (blue), $\delta^{15}\text{N}_{\text{PON}}$ (green), $\delta^{18}\text{O}_{\text{NO}_3}$ (dark blue), $\Delta^{17}\text{O}_{\text{NO}_3}$ (magenta)). Concentrations tested range from 1 – 9 $\mu\text{moles L}^{-1}$. Oxygen isotopes appear insensitive to ammonium concentrations, while N isotopologues are mildly sensitive (1-2‰).

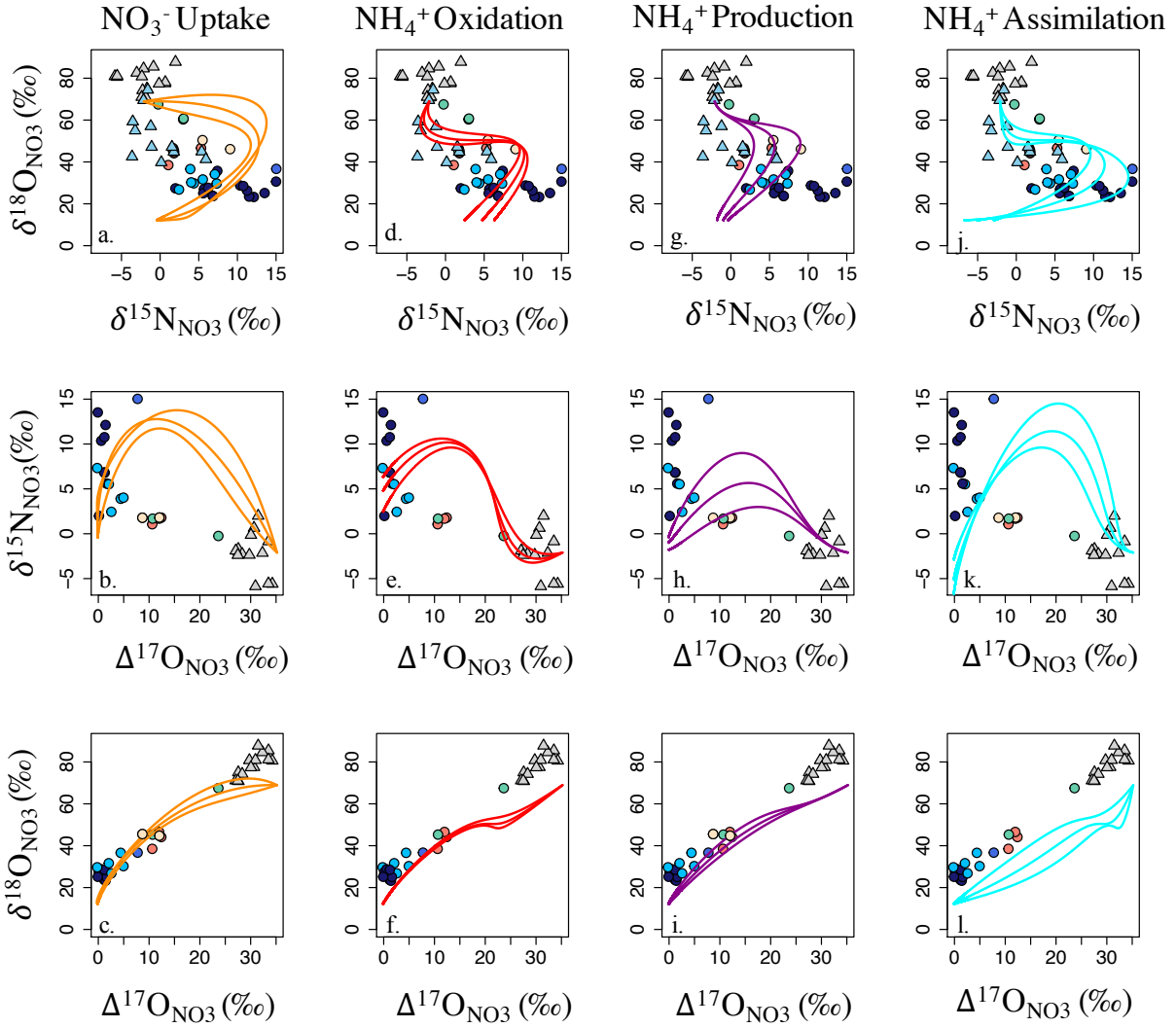


Figure S9. Sensitivity of property-property plots to biological rates: a-c.) nitrate uptake (model output = orange tested at 0.2, 0.4, 0.8 days⁻¹), d-f.) ammonium oxidation (model output = red tested at 0.2, 0.4, 0.8 days⁻¹), g-i.) ammonium production (model output=purple tested at 0.02, 0.04, 0.08 days⁻¹) and j-l.) ammonium assimilation (model output = blue tested at 0.2, 0.4, 0.8 days⁻¹). All modeled output reflects values from initiation to steady-state, are based on the “Atm-Only” model, parameterized with mean atmospheric observations from this study, and compared to actual observations.

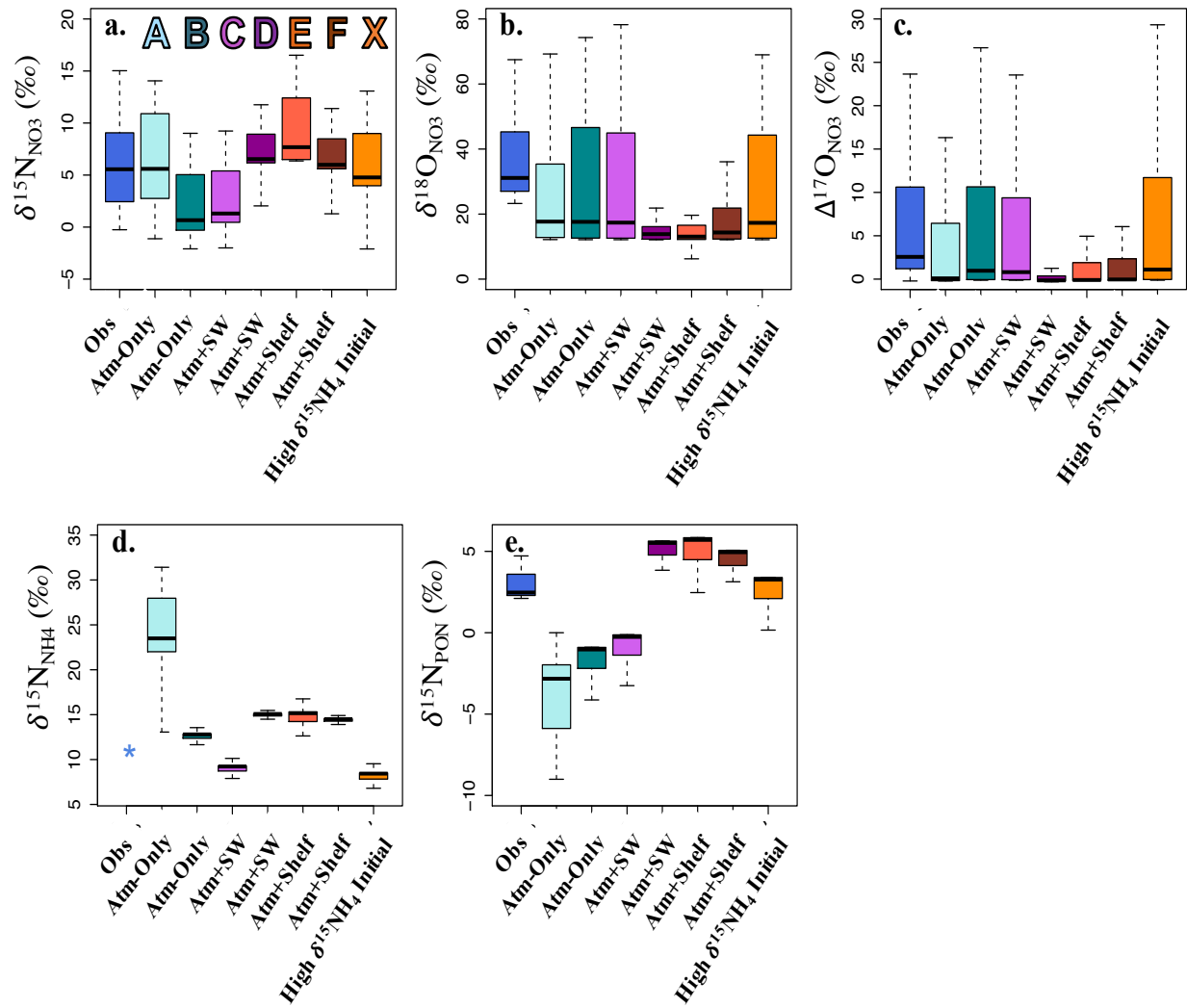


Figure S10: Median and range of parameter values obtained via respective scenarios (A-X) for isotopologue evolution in sea ice compared to sea ice observations (Obs). The top and bottom of each box represent the 25th and 75th percentiles, the distances between reflect the interquartile ranges, and the black line inside each box is the sample median. Whiskers extending above and below each box capture the furthest observations within the whisker length. The blue asterisk reflects a single observation.

Isotope	IAEA-N ₃ (n)	USGS34 (n)	USGS35 (n)	Sample Replicates (n*)
$\delta^{15}\text{N}$	0.21 (22)	0.29 (16)	0.24 (21)	0.23 (6)
$\delta^{18}\text{O}$	0.72 (22)	0.74 (16)	0.73 (21)	0.44 (6)
$\Delta^{17}\text{O}$	-	0.62 (14)	0.92 (15)	0.85 (4)

Table S1. Pooled standard deviation (1σ) of isotopic reference materials for all sample analyses where n is number of analyses and n* represents the number of pairs.

Latitude	$\delta^{15}\text{N}_{\text{NH}_4^+}$ (n)	StdDev
87	11.71 (3)	1.45
85	2.98 (2)	0.80
88	3.90 (3)	0.42

Table S2. Mean $\delta^{15}\text{N}_{\text{NH}_4^+}$ values for 1 sea ice (station at 87°N) and 2 snow samples (stations at 85°N and 88°N) collected during GEOTRACES Arctic cruise at stations located at 87°N, 85°N and 88°N. Each sample was analyzed (n) times to quantify reproducibility.

Process	Rate (d ⁻¹)	¹⁵ ϵ (‰)	¹⁸ ϵ (‰)
NO₃⁻ uptake	0.1-0.6	5-10	5-10
NH₄⁺ uptake	0.001-0.1	0	—
PON ammonification	0.02-0.03	0	—
NH₄⁺ oxidation	0.001-0.3	19 – 30	*, **

*Isotope effect (13.5 ‰) for equilibration between NO₂⁻ with H₂O

** Isotope effect (15‰) for O-atom incorporation from H₂O during oxidation of NO₂⁻ to NO₃⁻

Table S3: Ranges of values of N-cycling rates and corresponding isotope effects tested in the model scenarios as discussed in Sections 2.4.1 and Supplementary Text S3 (see also Fig. 2).

Framework	Parameter	Description	Scenario Values Tested			Reference
			Atm Only, High $\delta^{15}\text{N}_{\text{atm}}$ Initial (A-B,X)	Atm + SW (C, D)	Atm + Shelf (E, F)	
Model	dt	Time step	0.01 days	0.01 days	0.01 days	
	$\alpha^{15}\text{NO}_3^-$ uptake	N isotope effect of NO_3^- assimilation	1.010	1.010	1.010	Difiore et al. 2009
	$\alpha^{18}\text{NO}_3^-$ uptake	O isotope effect of NO_3^- assimilation	1.010	1.010	1.010	Difiore et al. 2009
	α^{15} nitrification	N isotope effect of NH_4^+ oxidation/nitrification	A (1.030), B,X (1.019)	1.019	1.019	Casciotti et al. 2003
	$\alpha^{15}\text{NH}_4^+$ uptake	N isotope effect of NH_4^+ uptake	1.000	1.000	1.000	Hoch et al. 1992; Liu et al. 2013
	$\alpha^{18}\text{N}_2\text{O}$	O isotope effect for incorporation of water oxygen during oxidation from nitrite to nitrate	1.015	1.015	1.015	Buchwald and Casciotti 2010
	$\alpha^{18}\text{eq}$	O isotope effect for H_2O - NO_2^- equilibration	1.0135	1.0135	1.0135	Buchwald and Casciotti 2013
	α^{15} ammonification	N isotope effect during ammonification	1.000	1.000	1.000	Lehmann et al. 2002
Rates	NO_3^- uptake	Uptake rate of NO_3^-	A (0.6 days ⁻¹), B,X(0.1days ⁻¹)	0.1 days ⁻¹	0.1 days ⁻¹	
	NH_4^+ oxid	Oxidation rate of NH_4^+	A (0.3 days ⁻¹), B,X(0.1 days ⁻¹)	0.1 days ⁻¹	E (0.1 days ⁻¹); F (0.001 days ⁻¹ ; 0.1 days ⁻¹)	
	NH_4^+ prod	Production rate of NH_4^+	A (0.03 days ⁻¹), B,X(0.02days ⁻¹)	0.02 days ⁻¹	0.02 days ⁻¹	
	NH_4^+ assim	Assimilation rate of NH_4^+	A (0.08 days ⁻¹), B,X(0.1 days ⁻¹)	0.1 days ⁻¹	E (0.1 days ⁻¹); F (0.001 days ⁻¹ ; 0.1 days ⁻¹)	
Nutrients	$[\text{NO}_3^-]$ initial	Initial $[\text{NO}_3^-]$	A (2.5 μM), B,X (1.7 μM)	C (1.6 μM), D (0.4 μM) *C (1.2 μM), D(0.3 μM)	E (11.7 μM), F (10.0 μM ; 1.9 μM)	This study**
	$[\text{NH}_4^+]$ initial	Initial $[\text{NH}_4^+]$	A (2.5 μM), B,X (1.0 μM)	C (1.2 μM), D (2.7 μM)	E (5.0 μM), F (4.0 μM ; 10.0 μM)	This study**
Isotopologues	$\delta^{15}\text{N}_{\text{NO}_3^-}$ initial	Initial $\delta^{15}\text{N}$ NO_3^-	A (0‰), B,X (-2.1‰)	C (-2.0‰), D (3.4‰) *C (-1.1‰), D (4.2‰)	E (6.5‰), F (8.0‰; 0.1‰)	This study**
	$\delta^{15}\text{N}_{\text{NH}_4^+}$ initial	Initial $\delta^{15}\text{N}$ NH_4^+	A (0‰), B (3.4‰), X (15‰)	C (4.3‰), D (6.9‰)	E (7.1‰), F (8.0‰; 7.0‰)	This study**
	$\delta^{18}\text{O}_{\text{NO}_3^-}$ initial	Initial $\delta^{18}\text{O}$ NO_3^-	A (82‰), B,X (73.9‰)	C(77.8‰), D(35.9‰) *C (66.1‰), D (27.8‰)	E (10.9‰), F (0.0‰; 70.1‰)	This study**
	$\Delta^{17}\text{O}_{\text{NO}_3^-}$ initial	Initial $\Delta^{17}\text{O}$ NO_3^-	A-X (30.4‰)	C (29.9‰), D (13.8‰) *C (29.8‰), D (12.5‰)	E (4.5‰), F (0.0‰; 27.8‰)	This study**

* Trials where the combined average of the aerosols and snow were used to initialize the model

** Shelf scenario references used to inform model: Morales et al. 2014; Granger et al. 2011, 2018; Brown et al. 2015b; Fripiat et al. 2018

Table S4. Parameters used and values selected for each model scenario tested.

Initialization	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{18}\text{O}_{\text{NO}_3}$	$\Delta^{17}\text{O}_{\text{NO}_3}$	$\delta^{15}\text{N}_{\text{NH}_4}$	$\delta^{15}\text{N}_{\text{PON}}$
* NH_4^+ : 1 μM , $\delta^{15}\text{N}_{\text{NH}_4}$: 3.4‰	-2.1 to 9.0‰ (2.4‰)	12.1 to 68.9‰ (27.7‰)	-0.1 to 32.3‰ (6.8‰)	3.4 to 13.0‰ (8.5‰)	-6.3 to 0.0‰ (-1.8‰)
NH_4^+ : 1 μM , $\delta^{15}\text{N}_{\text{NH}_4}$: 12‰	-2.1 to 12.0‰ (5.3‰)	12.1 to 68.9‰ (27.8‰)	-0.1 to 35.2‰ (6.8‰)	10.2 to 20.2‰ (12.3‰)	-3.2 to 2.2‰ (1.3‰)
NH_4^+: 1 μM, $\delta^{15}\text{N}_{\text{NH}_4}$: 15‰	-2.1 to 13.1‰ (6.4‰)	12.1 to 68.9‰ (27.7‰)	-0.1 to 35.3‰ (6.8‰)	11.4 to 22.9‰ (13.7‰)	-2.0 to 3.4‰ (2.5‰)
NH_4^+ : 10 μM , $\delta^{15}\text{N}_{\text{NH}_4}$: 3.4‰	-6.5 to 8.0‰ (3.1‰)	12.1 to 68.9‰ (17.1‰)	-0.1 to 35.2‰ (2.1‰)	3.4 to 18.5‰ (11.8‰)	-0.1 to 1.8‰ (1.3‰)
NH_4^+: 4 μM, $\delta^{15}\text{N}_{\text{NH}_4}$: 8‰	-2.6 to 11.4‰ (6.0‰)	12.1 to 68.9‰ (21.0‰)	-0.1 to 35.2‰ (3.8‰)	8.0 to 21.2‰ (14.2‰)	0 to 4.1‰ (3.5‰)
NH_4^+ : 4 μM , $\delta^{15}\text{N}_{\text{NH}_4}$: 12‰	-2.1 to 14.1‰ (8.7‰)	12.1 to 68.9‰ (21.0‰)	-0.1 to 35.2‰ (3.8‰)	12.0 to 24.9‰ (17.2‰)	0 to 6.9‰ (6.3‰)
NH_4^+ : 4 μM , $\delta^{15}\text{N}_{\text{NH}_4}$: 15‰	-2.1 to 16.2‰ (10.7‰)	12.1 to 68.9‰ (21.1‰)	-0.1 to 35.2‰ (3.8‰)	15.0 to 27.7‰ (19.5‰)	0 to 9.0‰ (8.4‰)

Table S5. Model sensitivity to changes in ammonium concentration and isotopic composition at scenario initialization. Bold outputs highlight initialization conditions that match multiple isotopes.

Chapter Three

Quantifying $\Delta^{17}\text{O}$ of nitrate in low concentration samples via isotope dilution

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In Prep for *Rapid Communications in Mass Spectrometry*

Abstract

Characterization of $\Delta^{17}\text{O}$ presents interesting insights from atmospheric and ecosystem perspectives. Sample requirements needed to characterize $\Delta^{17}\text{O}$ of nitrate typically range from 50-200 nmols. Samples with very low concentrations ($<1\text{ }\mu\text{M}$) or limited sample are often pre-concentrated which can compromise sample results (e.g., isotopic fractionation). We present a new approach that removes sample pre-treatment and allows for quantification of $\Delta^{17}\text{O}$. Using the denitrifier method, NO_3^- is converted to N_2O gas, which is then measured via continuous flow on a Gas Chromatograph Isotope Ratio Mass Spectrometer (Sigman et al., 2001; Casciotti et al., 2002; Kaiser et al., 2007). An isotope dilution technique was developed to allow for measurement of very low concentration samples (e.g. $<0.4\text{-}0.6\text{ }\mu\text{M}$) and evaluated using reference materials and previously measured samples as a proof of concept. This technique involves the addition of a known reference material to an aliquot of sample to meet analytical mass requirements followed by mass balance to obtain the $\Delta^{17}\text{O}$ composition of the unknown sample. Isotope dilution presents an alternative method for meeting all analytical requirements of $\Delta^{17}\text{O}$ characterization. Using a calibration between mass spectrometer peak area and mass injected, the $\Delta^{17}\text{O}$ is quantified for a variety of samples with an uncertainty of $\pm 10\text{-}15\%$ uncertainty ($\sim 2\text{-}3\text{‰}$) for natural samples. This technique can reliably constrain the area-mass relationship required to determine $\Delta^{17}\text{O}$ of nitrate in low concentration samples. While the precision is higher than other approaches, this method reduces the risk of compromising sample results due to pre-treatment and allows for the determination of low concentration samples with limited volume.

1. Introduction

Less abundant oxygen isotopes (^{17}O and ^{18}O) fractionate in a predictable way via most chemical and physical processes in terrestrial materials (e.g., atmospheric O_2 , atmospheric water, seawater, bulk silicate) as a function of mass differences. This mass-dependent behavior describes the fractionation of $\delta^{17}\text{O}$ relative to $\delta^{18}\text{O}$ and is defined as: (1) $\delta^{17}\text{O} = 0.52 \times \delta^{18}\text{O}$ (Matsuhisa et al., 1978). Oxygen anomalies were first recognized in meteorites (e.g., Clayton et al., 1973) and ozone, which diverge from this mass-dependent behavior resulting in ^{17}O anomalies (Thiemens and Heidenreich, 1983). Ozone (O_3) is an important oxidant in the atmosphere, thus these ^{17}O anomalies are transferred during any oxidation reaction involving O_3 , making them particularly useful for atmospheric chemistry studies because they are distinctive of photochemical reactions and their characterization has become more extensive in the recent past (Miller et al., 2002; Michalski et al., 2003; Thiemens et al., 1999).

One such example results from interactions between nitrogen oxides ($\text{NO}_x = \text{NO} + \text{NO}_2$) and O_3 , where atmospheric nitrate has distinctly elevated $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$, which can be defined non-linearly and linearly as $\Delta^{17}\text{O}$ (Miller et al., 2002; Kaiser et al., 2007; Michalski et al., 2003). For consistency with previous work, $\Delta^{17}\text{O}$ is characterized using the linear definition ($\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O} \neq 0$), which describes the average mass-dependent covariation of the less abundant isotopes found in terrestrial materials (Thiemens and Heidenreich, 1983; Thiemens et al., 1999; Miller et al., 2002; Michalski et al., 2003). This relationship describes the anomalous ^{17}O present in trace gases relative to ^{18}O , which can result in $\Delta^{17}\text{O}$ ranging from 22-44‰ from mid- to high-latitudes and deviates from the expected mass-dependent relationship (1) (Michalski et al., 2012 and references therein). With the exception of O_3 , other atmospheric oxidants (RO_2 , O_2 , OH and H_2O) have $\Delta^{17}\text{O}$ close to 0‰ (Michalski et al., 2004; 2012 and

references therein), thus this mass-independent anomaly acts as a conservative tracer of atmospheric nitrate and has been used for understanding changes in oxidation chemistry and oxidizing capacity of the atmosphere across space and time using field observations and modeling studies (e.g., Geng et al., 2014, 2017; Kunasek et al., 2008; Fibiger et al., 2013; Alexander et al., 2009, 2019), as well as, gaining insight into the role of atmospherically oxidized nitrate in aquatic and terrestrial environments (e.g., Michalski et al., 2004; Hundey et al., 2016; Bourgeois et al., 2018). However, this atmospheric feature ($\Delta^{17}\text{O}$) can be quite challenging to characterize.

To determine $\Delta^{17}\text{O}$, both chemical and bacterial methods can be used to convert sample NO_3^- to N_2O gas followed by isotopic characterization of O_2 via microwave discharge (Mukotaka et al., 2013), cavity ringdown laser spectroscopy (Gázquez and Claire, 2018), or pyrolysis and isotope ratio mass spectroscopy (Kaiser et al., 2007; Komatsu et al., 2008; Geng et al., 2013). A disadvantage of most methods is that they have large sample size requirements (e.g., 50-300 nmols, where 100 nmols is most common) to generate reproducible results (Kaiser et al., 2007; Morin et al., 2009; Geng et al., 2013). This often acts as a limiting step for the convenient analysis of low concentration samples ($<1\ \mu\text{M}$) and depending on sample type, collection volume can also be a limitation often prohibiting analysis entirely. Both challenges are often incurred during ice core research. The bacterial denitrifier method allows for much smaller sample sizes (e.g., 30-50 nmols), but samples with low concentrations or limited volume must be pre-treated. Concentration methods have been implemented to achieve adequate mass requirements while adhering to analytical volume constraints when conducting isotopic analysis (Silva et al., 2000; Frey et al., 2009; Erbland et al., 2013; Shi et al., 2015). Pre-treatment of samples can lead to fractionation of the isotopic composition and introduce matrix blanks from

concentrating samples via resin columns that are not always consistent and easily correctable (Shi et al., 2015). Specifically, a two-step correction has been used to account for the influence of an inconsistent NO_3^- blank detected among different batches and lot numbers of NaCl salts used for sample pre-treatment (see Shi et al., 2015 for details). Consequently, it is ideal that isotopic analysis take place without pre-treating samples to avoid these issues.

As an alternative approach, we introduce a new application of the denitrifier-pyrolysis method using isotope dilution to circumvent situations where pre-treatment of samples may be required. Isotope dilution is the quantitative addition of an isotopic spike to a sample of some unknown isotopic composition, where the composition of that mixture is measured using an isotope ratio mass spectrometer, and the isotopic composition of the unknown is calculated using an isotope mass balance model (see Methods for further details) (Garcia Alonso and Rodriguez-Gonzalez, 2013; Hayes, 2004). This technique has been used for a variety of applications (typically radiogenic in nature) where trace amounts of an element/compound of interest are more easily quantifiable using this approach. We discuss a series of experiments used to validate this technique using the denitrifier method, and its application using standard mixes and real samples that have been previously characterized for $\Delta^{17}\text{O}$ of nitrate.

2. Methods

2.1 Overview of the denitrifier-pyrolysis method for $\Delta^{17}\text{O}$ analysis

Previous work describes protocols implemented for $\Delta^{17}\text{O}$ analysis at Brown (e.g., Fibiger et al., 2013; Buffen et al., 2014; Shi et al., 2015), but for completeness we describe the method here. The characterization of $\Delta^{17}\text{O}$ requires the measurement of $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$, which are quantified using the bacterial denitrifier method and gold-tube pyrolysis (Sigman et al., 2001, Casciotti et al., 2002, and Kaiser et al., 2007). Discussion of medium preparation, ideal culturing

conditions and sample preparation are detailed in the original methods papers (Sigman et al., 2001; Casciotti et al., 2002). An aliquot of sample containing 35-50 nmols of nitrate is quantitatively converted to nitrous oxide (N₂O) using denitrifying bacteria (*Pseudomonas chlororaphis* spp. *aureofaciens* (ATCC® 13985™, Manassas, VA)), which is then extracted, purified and cryo-focused before thermal decomposition to N₂ and O₂ in a gold tube heated to 770°C (Kaiser et al., 2007; Fibiger et al., 2013). The O₂ is measured at m/z 32, 33, and 34 on a gas chromatograph isotope ratio mass spectrometer (GC-IRMS, Thermo-Scientific Finnigan Gas Bench II and Delta V Plus) in continuous flow mode. Nitrate isotopic NIST reference materials, USGS34 (KNO₃) and USGS35 (NaNO₃), are prepared in ultrapure MilliQ (18.2 Ω) water (i.e., MQWA) and analyzed in each set to mimic sample processing that takes place, along with a mix of USGS34:USGS35 as an internal quality control. The measured mass ratios are corrected based on a linear fitting of reference materials to known values for USGS34 ($\Delta^{17}\text{O} = -0.292\text{‰}$) and USGS35 ($\Delta^{17}\text{O} = 21.6\text{‰}$) (Kaiser et al., 2007). Isotopes are corrected for bacteria and water blank effects, exchange with water, and mass-dependent fractionation following methods in Kaiser et al., (2007). Isotope ratios are reported in delta notation defined as: $\delta (\text{‰}) = [(R_{\text{sample}})/(R_{\text{standard}})-1] \times 1000$, where $R = {}^{17}\text{O}/{}^{16}\text{O}$ and ${}^{18}\text{O}/{}^{16}\text{O}$ for $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$, respectively and values are referenced to Vienna Standard Mean Ocean Water (VSMOW, Böhlke et al., 2003; Kaiser et al., 2007). $\Delta^{17}\text{O}$ is defined linearly ($\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O} \neq 0$) to maintain consistency with previous work (e.g., Michalski et al., 2002, 2003, 2004; Alexander et al., 2004, 2009; Kunasek et al., 2008; Frey et al., 2009; Erbland et al., 2013; Fibiger et al., 2013; Buffen et al., 2014; Shi et al., 2015).

2.2 Calibration of a nitrate reference spike

Normalizing the total volume for each injection minimizes any possible volume effect and using pre-purged water minimizes non-sample N₂O from being analyzed with sample gas. For this study, volumes of helium-purged MQWA/sample water were standardized to 15-30 mL, such that maximum volume did not exceed 20 and 35 mL (e.g., sample, bacteria, and spike) for respective experiments. Experiments were initially designed for 30 mL of volume, but we found a greater sensitivity and higher area yield when injections were reduced to 15 mL. We present data from both experimental setups, but emphasize results from experiments conducted using 15 mL. We refer to 30 mL and 15 mL experimental conditions as Experiments 1a-e, and 2a-d, respectively.

During each run, a calibration is generated relating a range of masses of USGS34 to volt-seconds on a GC-IRMS. We chose USGS34 as the spike because sensitivity tests suggest that using a standard as the spike furthest from the anticipated isotopic composition produces the best results (see Table 1 for sensitivity calculations). For example, USGS34 ($\Delta^{17}\text{O} = -0.292\text{‰}$) acts as a more sensitive spike than USGS35 ($\Delta^{17}\text{O} = 21.6\text{‰}$) for samples that are predicted to reflect atmospheric nitrate, which typically range from 22-40‰, (Michalski et al. 2012; Fibiger et al. 2013). For the purpose of this study and based on these results, USGS34 was selected as the spike for all experiments discussed below and we set a lower limit of 30 nmols of nitrate as analytically measurable, which was based on previous determination of mass spectrometer detection limits using the $\Delta^{17}\text{O}$ method (Buffen et al., 2014; Shi et al., 2015).

USGS34 Calibration			USGS35 Calibration	
Sample (nm)	USGS34 (nm)	$\Delta^{17}\text{O}$ Mix (‰)	USGS35 (nm)	$\Delta^{17}\text{O}$ Mix (‰)
5	30	4.0	30	28.8
10	30	7.3	30	27.9
15	30	9.8	30	27.2
20	30	11.8	30	26.6
25	30	13.5	30	26.2
30	30	14.9	30	25.8
Difference (‰)		10.8	3.0	

Table 1: Mass balance sensitivity calculations for two reference standards (e.g., USGS35 $\Delta^{17}\text{O}$ = 21.6 and USGS34 $\Delta^{17}\text{O}$ = -0.292) assuming the “sample” has an isotopic composition ($\Delta^{17}\text{O}$) of 30‰.

Constraining the relationship between sample mass (nmols nitrate) and the corresponding signal registered by the mass spectrometer (volt-seconds) is critical. This relationship is then used to determine the mass of unknown samples by difference (i.e., sample+spike mixture – spike = sample). A mass calibration was conducted for a range of masses during each analytical run (e.g., 30-60 nmols of analyte), where triplicate injections were conducted for each mass of the selected spike standard. MQWA was pre-purged with helium and added to each calibration vial at a normalized volume as described above. Instrument sensitivity and healthy bacteria are critical for generating a strong linear relationship between mass injected (i.e., nmols) and peak area yield (i.e., volt-seconds) (Sigman et al., 2001; Casciotti et al., 2002).

2.3 Experimental setup & dilution calculations

Spike injections were normalized to 30 nmols of a high concentration (50 $\mu\text{mol L}^{-1}$) USGS34 solution, which ensured that all analyses had the minimum mass required for accuracy and precision once the low mass sample was added. A standardized volume of sample was then added to each vial (i.e., 15 mL). Each analytical set included: a bacteria blank, a MQWA blank of equivalent volume to samples, a lab quality control standard (30 nmols of 34:35 Mix), and a

second nitrate reference standard (30 nmols of USGS35), which were used for correction purposes.

Once the experimental procedures were complete, we conducted a series of calculations to determine/characterize the $\Delta^{17}\text{O}$ composition of the “unknown” sample. First, a linear regression ($y = mx + b$) was generated from the calibration curve relating mass injected (x) and peak area yield (y) with a corresponding slope (m) and y-intercept (b), which describes the generated peak area for different mass additions of the spike (USGS34). If this is well constrained, the same relationship can be used to decipher the area of an unknown sample in the spike-sample mixture. Using this linear relationship, the residual mass of the added unknown sample in the spike-sample mixture was determined by: 1) taking the difference between the average area for triplicate 30 nmol injections of the spike (USGS34) and the mixture area, and 2) substituting the residual area (RA) into the linear regression generated from the calibration curve to solve for the residual area mass (RA Mass) illustrated by the equations below:

$$(1) \text{ Residual Area (RA)} = \text{Mix Area} - \text{Average Area (30 nmol USGS34)}$$

$$(2) \text{ Residual Area Mass (RA Mass)} = (\text{RA} - b)/m$$

Once isotopic corrections were made, the isotopic composition of the mixture (e.g., spike + sample) and spike, and the mass of the mixture, sample, and spike were used to solve for the $\Delta^{17}\text{O}$ of the sample by mass balance using equation (3). Equation 3 is based on and adapted from ($\delta_{\text{sample}} = (\delta_{\text{mix}}(M_{\text{spike}} + M_{\text{sample}}) - (\delta_{\text{spike}} \times M_{\text{spike}})) / M_{\text{sample}}$) (Hayes, 2004).

$$(3) \Delta^{17}\text{O}_{\text{sample}} = \frac{\Delta^{17}\text{O}_{\text{Mix}}(M_{\text{spike}} + M_{\text{sample}}) - (\Delta^{17}\text{O}_{\text{spike}} \times M_{\text{spike}})}{M_{\text{sample}}}$$

An outline schematic of these steps is illustrated in Figure 1.

Generate calibration regression of the spike relating a range of masses to area ($y = mx + b$, See Figure 2)

Test spike-standard mixtures (spike + standard)
(See Table 3)

Calculate (1) residual area (RA) by difference and (2) residual area mass (RA Mass) using calibration regression:

(1) $RA = \text{Mix Area} - \text{Average Area (30 nmol spike)}$
(2) $RA \text{ Mass} = (RA - b)/m$

Calculate $\Delta^{17}O$ of sample using mass balance eqn (3):

$$\Delta^{17}O_{\text{sample}} = \frac{\Delta^{17}O_{\text{Mix}}(M_{\text{spike}} + M_{\text{sample}}) - (\Delta^{17}O_{\text{spike}} \times M_{\text{spike}})}{M_{\text{sample}}}$$

Figure 1: Schematic of dilution method major steps.

2.4 Standard mixes & environmental samples used for method validation

We take two approaches to test the validity of the dilution approach. Preliminary experiments using nitrate reference standards were conducted followed by analysis of previously characterized environmental samples. First, the peak area of USGS34 was calibrated from 30-60 nmols, where each mass was injected in triplicate. Then, 30 nmols of USGS34 was added to fifteen test “sample” vials. Using a USGS35 solution (e.g., 50 μM for this study), triplicate injections of 10, 15, 20, and 30 nmols were added to each spiked “sample” vial followed by 15 mL of pre-purged MQWA (30 mL for Exp: 1a-e), which covered the calibration range (e.g., 40 – 60 nmols). In theory, the mixtures should reflect a $\Delta^{17}O$ that is intermediate between both reference values according to the mass of USGS35 added to each mix and the $\Delta^{17}O$ of USGS35 (21.6‰) (measured as an unknown) determined via mass balance. Each standard mixture was used as a quality control to check whether the dilution experiment was successful, based on

whether the isotopic composition of the “unknown” (i.e., USGS35 in this case) met a threshold within the expected $\Delta^{17}\text{O}$.

Previously measured samples were selected for reanalysis and validation experiments. High concentration samples (as high as 99 μM) from two separate data sets (Princeton rainfall (Kaiser et al., 2007) and Colorado lake water/precipitation (Clark et al., in prep) and two separate analytical periods (circa 2003 and 2018, respectively) were selected, diluted to concentrations less than 1 μM and treated as unknowns. Three samples from each dataset were injected in triplicate. As described in the previous experiment, the same calibration range was implemented and 30 nmol of spike was added to 15 mL or 30 mL of sample. To verify that the change in standardized volume did not dramatically alter the isotopic composition determined via this method, the Princeton samples were analyzed under both experimental conditions (i.e., 15 and 30 mL injections). Additionally, a selection of samples not previously characterized were analyzed multiple times (on at least two separate occasions in triplicate) to assess reproducibility across analytical experiments and bacterial batch conditions.

3. Results

Experimental results are described for: 30 mL (Exp. 1a-1e) and 15 mL (Exp. 2a-2d) calibration experiments followed by the application to pre-made standard mixtures and environmental samples. Uncertainty results are also reported.

3.1 Mass-area calibration sensitivity of the nitrate reference spike

Calibrations for mass injections of USGS34 were relatively consistent and reproducible between runs (Figure 2; Table 2). Regression slopes ranged from 0.38 - 0.65 with an R^2 of 0.98 or better (see Figure 2; Table 2). Slope sensitivity was higher (~ 0.6) for 15 mL experiments (2a-d) compared to larger injections (~ 0.4 for 30 mL, Exp. 1a-e) (Figure 2; Table 2). The regression

slopes (~ 0.6) correlate to higher yield of N_2O via bacterial conversion of nitrate, which are important to the sensitivity of the residual area and mass calculations. The precision of the 30 nmol of USGS34 spike added to all samples was quantified by taking the standard deviation of triplicate injections, which on average was better than 0.4 volt-seconds. The average area for the standardized spike addition (30 nmols USGS34) was $\sim 17.1 \pm 1.2$ volt-seconds (across Exp. 2a-d). The mass-peak area relationship was linear with reproducible peak area yields for 10 nmol differences in mass injected, on average 6.26 ± 0.91 volt-seconds were observed between calibration points (Figure 2). Since the areal yield is dependent on bacteria health and mass spectrometer sensitivity, an average value for the spike was calculated for each run and used to calculate the residual area of the unknown mass in all standard and sample mixtures (Equation 1).

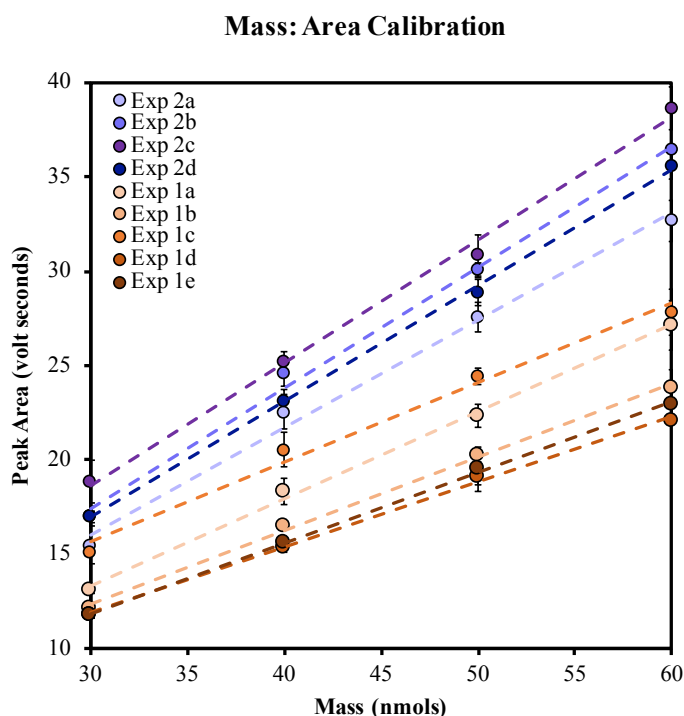


Figure 2: Calibration curves using USGS34 for Experiments 2a-d (15 mL experiments) and 1a-e (30 mL experiments).

Experiment	Normalized Volume (mL)	Slope (m)	Y-intercept (b)	R ²	Spike Area 30 nmol (x±1σ)	Relative Standard Deviation (%)
2a	15	0.5687	-1.0396	0.9923	15.41±0.33	2.14
2b	15	0.6374	-1.6743	0.9960	16.99±0.30	1.77
2c	15	0.6508	-0.915	0.9958	18.83±1.14	6.05
2d	15	0.6144	-1.4928	0.9990	17.02±0.49	2.88
1a	30	0.4617	-0.5615	0.9975	13.08±0.12	0.92
1b	30	0.388	0.7066	0.9977	12.12±0.27	2.23
1c	30	0.4205	3.0417	0.9878	15.09±0.63	4.17
1d	30	0.3472	1.4591	0.9975	11.76±0.11	0.94
1e	30	0.3737	0.6374	0.9990	11.75±0.14	1.19

Table 2: Linear regression components and spike yield for each experiment calibration conducted.

3.2 Method validation using standard mixes & environmental sample mixes

As expected, a gradual shift in the measured isotopic composition of the mixtures toward a higher $\Delta^{17}\text{O}$ was observed as mass additions of 10, 15, 20 and 30 nmols of USGS35 were added (not shown). Dilution experimental success was based on whether the isotopic composition of the “unknown” (i.e., USGS35 in this case) within the standard mixtures was within 10-15% (2-3‰) of the expected $\Delta^{17}\text{O}$. However, mass balance calculations across all mixture injection replicates reflected an average of $23.4 \pm 4.0\%$ or 16.9% uncertainty for USGS35 in the 15 mL experiments (e.g., reference value $\Delta^{17}\text{O} = 21.6\%$). There is inherently greater uncertainty (16.9%) associated with the standard mixes due to the additional step of pipetting small mass amounts of USGS35 to these mixtures (Figure 3; Table 3). In contrast, we determine uncertainty for the actual unknowns based on the 30 nmol spike injections. A lower uncertainty is associated with the unknown samples because they only include the spike (0.6 mL of USGS34) and sample (15 mL) compared to the standard mixes, which include small mass

injections of the spike (0.6 mL of USGS34) and a second additional standard (0.2 to 0.6 mL of USGS35) in addition to MQWA. Bacteria blanks were undetectable (<0.2 volt-seconds), as were water blanks for all but two experiments.

With the exception of one of these samples, all $\Delta^{17}\text{O}$ values characterized by dilution were within 3‰ of the previously characterized samples from Princeton and Colorado (Table 4). Validation samples tested at both standardized volumes (i.e., 15 and 30 mL, Princeton rain, n=3) generated reproducible isotopic results that were within the overall uncertainty regardless of the change in volume (Table 4). For samples (CO-7 to CO-18) that were analyzed multiple times across experimental sets, we observed good agreement between runs (Table 5). With the exception of a few samples, the majority have an uncertainty $\leq 10\text{-}15\%$ (2-3‰) (Table 5). Interestingly, two analytical sets (Exp 2b-c) used the same bacteria batch, but were harvested ~ a week apart and resulted in higher $\Delta^{17}\text{O}$ values for the second set (2c). The greater variability observed for these samples is most likely a function of the bacteria media age.

3.3 Quantitative measures for assessing uncertainty

To assess the uncertainty of our measurements, we use three quantitative measures from our experiments. First, the generation of a calibration curve with a given R^2 for each experimental set reflects uncertainty associated with mass injections at each calibration point (mass) and the corresponding mass spectrometer response (volt-seconds yield). The relationship between these two calibration components produced R^2 values better than 0.99 for Experiments 2a-d and better than 0.98 for all but one set in Experiments 1a-e (Table 2). In addition, the standard mixes included in every experiment inherently incorporate some uncertainty with small additions of USGS35 (10, 15, 20, 30 nmols) added to each mixture on top of the uncertainty associated with the instrumental response and 30 nmol injections of USGS34. In particular, 10

and 15 nmol injections were more variable in the final isotopic output ($\Delta^{17}\text{O}$) for the reference value of USGS35 (Table 3), yet the average $\Delta^{17}\text{O}$ calculated across all experiments is within 1‰ (22.7‰) of the expected reference value (i.e., USGS35 $\Delta^{17}\text{O}$ =21.6‰) (Table 3). This is likely a function of both the sensitivity of the mass balance calculation to determine the reference value and the accuracy with which we were able to add these small amounts of mass in a reproducible manner. Lastly, we attribute the overall uncertainty of the method to the reproducibility of the sample replicates reanalyzed during multiple experiments, which contain the standardized spike amount (30 nmol) and a sample volume added in milliliter (i.e., 15 mL) as opposed to microliter (e.g., 200-600 μL or 0.2-0.6 mL) quantities. Reanalyzed samples include both validation sample sets (Princeton rain and Colorado precipitation/lake water), as well as, low concentration samples reanalyzed across multiple dilution experiments (Table 4).

Experiment	USGS 35 Mass	Calculated $\Delta^{17}\text{O}$ (‰) $\bar{x} \pm 1\sigma$
2a	10	21.7 $\pm$ 0.4
	15	24.2 $\pm$ 1.8
	20	24.6 $\pm$ 1.9
	30	27.1 $\pm$ 0.9
2b	10	20.2 $\pm$ 3.6
	15	17.6 $\pm$ 2.5
	20	23.4 $\pm$ 2.0
	30	22.9 $\pm$ 0.8
2c	10	23.1 $\pm$ 4.3
	15	18.4 $\pm$ 7.6*
	20	24.3 $\pm$ 0.7*
	30	23.0 $\pm$ 1.5*
2d	10	20.0 $\pm$ 2.3
	15	24.2 $\pm$ 3.0
	20	23.4 $\pm$ 1.5
	30	25.0 $\pm$ 1.7

Table 3: Average and standard deviation ($\bar{x} \pm 1\sigma$) for the calculated $\Delta^{17}\text{O}$ for added mass (10, 15, 20, 30 nm) of USGS 35 for Experiments 2a-d. An asterisk reflects 2 replicates instead of 3. For reference, $\Delta^{17}\text{O}$ of USGS35 =21.6‰.

Sample	Analyzed	$\Delta^{17}\text{O}$ (‰) $\bar{x} \pm 1\sigma$	Prev. $\Delta^{17}\text{O}$ (‰) Reference
CO-1	2.25.19*	27.7 $\pm$ 0.8	28.2
CO-2	2.25.19*	5.1 $\pm$ 1.2	3.2
CO-3	2.25.19*	9.1 $\pm$ 0.5	8.3
PRN-1	5.29.19	31.5 $\pm$ 0.1	29.3
	2.26.19*	34.4 $\pm$ 0.5	29.3
	$\bar{x} \pm 1\sigma$	33.0$\pm$2.1	-
PRN-24	5.29.19	28.9 $\pm$ 1.7	28.4
	2.26.19*	29.8 $\pm$ 1.9	28.4
	$\bar{x} \pm 1\sigma$	29.4$\pm$0.64	-
PRN-27	5.29.19	29.2 $\pm$ 1.6	26.3
	2.26.19*	29.1 $\pm$ 1.2	26.3
	$\bar{x} \pm 1\sigma$	29.2$\pm$0.1	-

Table 4: Previously characterized $\Delta^{17}\text{O}$ values (Prev. $\Delta^{17}\text{O}$) from validation samples in comparison with reanalysis via dilution ($\Delta^{17}\text{O}$, $\bar{x} \pm 1\sigma$). $\Delta^{17}\text{O}$ values on a given analytical date reflect 2-3 replicates per sample. The average and standard deviation is calculated to reflect and account for variability in bacteria experimental sets. An asterisk reflects experiments where the dilution volume was standardized at 30 mL instead of 15 mL.

Sample	Analyzed	$\Delta^{17}\text{O}$ (‰) $\bar{x} \pm 1\sigma$
CO-4	5.29.19	30.8 $\pm$ 0.7
CO-5	5.29.19	26.5 $\pm$ 1.6
CO-6	5.29.19	28.2 $\pm$ 2.4
CO-7	8.18.19	30.7 $\pm$ 6.5
	8.23.19	34.3 $\pm$ 0.0
$\bar{x} \pm 1\sigma$	-	32.5$\pm$2.5
CO-8	8.18.19	30.8 $\pm$ 2.3
	8.23.19	37.8 $\pm$ 0.6
$\bar{x} \pm 1\sigma$	-	34.3$\pm$4.9
CO-9	8.18.19	1.2 $\pm$ 0.3
	8.23.19	2.1 $\pm$ 0.3
$\bar{x} \pm 1\sigma$	-	1.7$\pm$0.6
CO-10	8.18.19	27.4 $\pm$ 2.1
	8.23.19	39.7 $\pm$ 7.1
$\bar{x} \pm 1\sigma$	-	33.6$\pm$8.7
CO-11	8.18.19	32.3 $\pm$ 0.8
	8.23.19	38.8 $\pm$ 2.7
$\bar{x} \pm 1\sigma$	-	35.6$\pm$4.6
CO-12	8.18.19	34.2 $\pm$ 0.9
	8.23.19	39.4 $\pm$ 0.3
$\bar{x} \pm 1\sigma$	-	36.8$\pm$3.7
CO-13	8.18.19	21.9 $\pm$ 0.1
	10.10.19	27.0 $\pm$ 3.6
$\bar{x} \pm 1\sigma$	-	24.5$\pm$3.6
CO-14	8.18.19	32.1 $\pm$ 7.5
	8.23.19	34.3 $\pm$ 5.2
$\bar{x} \pm 1\sigma$	-	33.2$\pm$1.6
CO-15	8.18.19	25.2 $\pm$ 3.6
	8.23.19	31.0 $\pm$ 7.7
$\bar{x} \pm 1\sigma$	-	28.1$\pm$4.1
CO-16	5.29.19	11.7 $\pm$ 0.4
	8.18.19	8.7 $\pm$ 0.4
	10.10.19	9.6 $\pm$ 0.6
$\bar{x} \pm 1\sigma$	-	10$\pm$1.5
CO-17	5.29.19	8.6 $\pm$ 1.9
	8.18.19	8.4 $\pm$ 2.2
$\bar{x} \pm 1\sigma$	-	8.5$\pm$0.1
CO-18	5.29.19	7.0 $\pm$ 1.4
	8.18.19	14.2 $\pm$ 4.8
	8.23.19	5.1 $\pm$ 0.9
	10.10.19	4.9 $\pm$ 0.9
$\bar{x} \pm 1\sigma$	-	7.8$\pm$4.4

Table 5: $\Delta^{17}\text{O}$ determination for “unknowns” from different 15 mL experiments, where each $\Delta^{17}\text{O}$ value on a given analytical date reflects 2-3 replicates per sample. For all samples, the average and standard deviation is calculated to reflect and account for variability in bacteria experimental sets.

4. Conclusions

Low concentration nitrate samples ($<1\text{ }\mu\text{M}$) have been nearly impossible to characterize for $\Delta^{17}\text{O}$ without pre-treatment of samples, which is further challenged when concentrations are low and sample volume is limited. Isotope dilution presents an ideal approach to analyze these samples which minimizes steps that can introduce fractionation and blank effects associated with methods involving pre-treatment of samples. This approach enables the user to measure the isotopic composition of samples containing 6-10 nmol ($\sim 0.4\text{-}0.6\text{ }\mu\text{M}$) of mass in addition to a baseline isotopic reference material (in this case 30 nmol) allowing us to push the boundaries of and remain well within the instrumental limit of detection. Measured uncertainties (10-15% for samples, and slightly higher, 16.9%, for standard mixes due to the added step of small mass additions) are larger than traditional techniques with higher concentrations of nitrate. However, in many cases in which the absolute mass is low, but the geochemical signal is large, the relative uncertainty is acceptable because the data are still useful to our understanding of the system measured (e.g., Michalski et al., 2004; Riha et al., 2014; Hundey et al., 2016; Bourgeois et al., 2018; Clark et al., 2020; Clark et al., in prep). While this approach may be highly applicable for ice core studies, it can be implemented on any sample type where concentration is the limiting factor for conducting isotopic analysis, potentially enabling for further detailed analyses in environments where data are lacking/minimal. The inherent uncertainty associated with test mixtures containing USGS34:USGS35 is larger than that associated with the unknowns/ previously uncharacterized samples, which are only dependent on the reproducibility of the standardized spike addition. We emphasize that the successful application of this method heavily relies on thoroughly constraining the relationship between small mass differences and peak area yield established during the spike calibration. This is especially important for the standardized

spike amount added to all sample-spike mixtures because the proceeding calculations used for the determination of the residual area and associated mass rely on a reproducible signal, which is contingent on accuracy of the solution injection, and complete quantitative conversion of that nitrate to the sample N₂O via healthy bacteria. While $\Delta^{17}\text{O}$ characterizations can be conducted using concentration techniques, we warn against methods that can introduce fractionation and possible blank effects and instead suggest this isotope dilution approach as a useful alternative.

5. Acknowledgements

The authors report no conflicts of interest. This work was funded by the National Science Foundation (Grant Number: OCE-1433989 to M. H.). We would like to thank Claire Bekker and Ruby Ho for their assistance with the experimental setup and bacteria maintenance.

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Chapter Four

The changing nitrogen deposition landscape in the western U.S.:
what role does transport play?

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Abstract

Federally mandated air quality regulations have resulted in steep declines in NO_x emissions, and a shift towards a more reduced nitrogen (NH_3 as NH_4^+) deposition landscape, on average, across the United States. Surface water chemistry in Colorado alpine environments have shown clear indications of continuing to receive high inputs of reactive nitrogen, despite the national emissions and deposition changes. Here we investigate trends in inorganic nitrogen concentration data in the Colorado alpine over the last ~2 decades during which the national nitrogen landscape has changed dramatically. We aim to understand whether sites in the western U.S., and particularly alpine ecosystems, are indeed seeing less atmospheric reactive N input over time and whether these trends are driven primarily by changes in emissions or whether changes in transport are important for driving trends. In general, the western U.S. sites (including alpine stations) follow the national trends of dramatically decreased nitrate concentrations, while ammonium has gradually increased/remained constant over time annually and seasonally. However, there is a good deal of site-to-site variability in these overall trends. Utilizing one high elevation long-term monitoring site in Colorado as a case study, back-trajectory clustering analyses were investigated to understand the role of atmospheric transport in concentration and deposition trends. Concentrations were more variable and tended to be higher during spring and summer seasons, which were also coincident with more variable transport during these seasons. Over the ~20-year period, the role of westerly short-range transport from western Colorado/Utah gradually became more important making up almost 50% of the transport on 4-year time-scales. These results suggest additional monitoring/regulation of regional emission sources may be needed in the future immediately to the west/southwest of this region.

1. Introduction

Nationally, nitrate deposition has decreased dramatically in the U.S. since 1990 as a result of national emission standards on precursor nitrogen oxides ($\text{NO}_x = \text{NO} + \text{NO}_2$); however, atmospheric total dissolved inorganic nitrogen inputs have not declined due to increasing ammonia gas (NH_3) emissions and ammonium (NH_4^+) deposition inputs (Lloret and Valiela, 2016; Li et al., 2016; Fenn et al., 2018; Zhang et al., 2018). Fossil fuel combustion sources (namely cars) dominate the NO_x budget. Catalytic converter technology on newer vehicles have enabled major reductions in NO_x emissions to take place (Li et al., 2016). However, an unexpected slowdown in NO_x emissions in the past decade may reflect an increasing contribution from industrial, small-scale industrial “area”, and off-road mobile sources with a simultaneous decreasing contribution from on-road gasoline vehicles and decreases in diesel NO_x that have been slower than expected (Jiang et al., 2018). The $\text{NH}_3/\text{NH}_4^+$ budget largely originates from agricultural fertilizers and livestock waste (predicted to be $\geq 80\%$ of NH_3 emissions in the U.S.; Reis et al., 2009). A recent study determined that NH_3 emissions have been increasing by $\sim 2.61\%$ per year from 2002-2016 over the Midwestern U.S. (agricultural hotspot) and found high emission events to be associated with increasing soil temperatures and reduced precipitation, which are predicted to continue in the future (Warner et al., 2017). Additionally, major urban regions where vehicle fleets are centralized contribute larger sources of NH_3 than previously suggested by National Emission Inventories (NEI) (Fenn et al., 2018). Ironically, this urban vehicle NH_3 source results from inefficient vehicular catalytic conversion of NO_x (Fenn et al., 2018). These findings suggest that NH_3 is prominent in both agrarian and urban environments. Consequently, deposition of inorganic nitrogen in the United States has largely transitioned from a NO_3^- -dominated to an NH_4^+ -dominated depositional landscape, though this varies across

different regional and spatial scales (Du et al., 2014; Li et al., 2016; Zhang et al., 2018; NADP, 2019).

Nitrogen (N) is an important macronutrient in natural environments that is essential for primary production or the synthesis of biomass. However, most terrestrial and aquatic systems are sensitive to external N inputs, resulting in a variety of ecological impacts (e.g., soil acidification, biodiversity losses, and eutrophication of surface waters) from N loading via atmospheric deposition processes. This has led to the development of critical loads in federally protected areas like national parks, where notable changes have taken place in sensitive systems such as alpine lakes (e.g., Rocky Mountain National Park, ROMO – Williams and Tonnesson, 2000; Baron et al., 2011). A critical load describes the deposition level of an air pollutant where ecological impacts remain undiscernible in sensitive ecosystems and has been set to 1.0 to 3.0 kilograms of N per hectare for western lakes (Burns et al., 2008; Baron et al., 2011). Critical loads are based on wet deposition data (NO_3^- and NH_4^+), but considerable effort has been made to identify and refine the complete reactive N budget (e.g., dry deposition of NH_3) in ROMO with the objective of understanding sources supplying N to federally protected mountain landscapes and driving wet deposition patterns (Beem et al., 2010; Benedict et al., 2013a-b; Benedict et al., 2018; Gebhart et al., 2011; Gebhart et al., 2014; Thompson et al., 2015).

A series of campaigns have been conducted over variable time scales to refine reactive N budget uncertainties and prominent deposition pathways including: the Rocky Mountain Airborne Nitrogen and Sulfur campaign (RoMANS I, two 5-week campaigns in spring and summer of 2006), RoMANS II (November 2008-2009), and a 4-month study (July 7 – October 15, 2014) in coordination the Front Range Air Pollution and Photochemistry Experiment (FRAPPE) (Malm et al., 2009; Beem et al., 2010; Benedict et al., 2013a-b, Benedict et al., 2018).

Collectively, measurements made during these campaigns have included wet deposition (NH_4^+ , NO_3^- , organic N), dry gas phase (NH_3 , HNO_3 , NO_2 , NO), particulates (NO_3^- and NH_4^+), and more recently specific organic N species (e.g., PAN, alkyl nitrates) (Malm et al., 2009; Beem et al., 2010; Benedict et al., 2013a-b, Benedict et al., 2018). These campaigns concluded that wet inorganic N is the dominant deposition pathway for the total reactive N budget (54%) with NH_4^+ deposition being higher than NO_3^- followed by 37% as wet organic N and dry NH_3 deposition based on the RoMANS II year-long study (Beem et al., 2010; Benedict et al., 2013a-b). More recent constraints on the reactive N budget in ROMO confirm two key points. First, the role of wet inorganic N deposition continues to be the dominant contributor to the reactive N budget (making up 85% of total budget based on FRAPPE measurements), with wet deposition of NH_4^+ making up the majority of wet inputs (49%) followed by NO_3^- (34%), and organic N (2% = 1.6% (PAN), 0.06% (alkyl nitrates)) (Benedict et al., 2018); and second, dry deposition comprises 15% of the total budget and is dominated by NH_3 gas (57%); Benedict et al., 2018). Constraining the total reactive N budget is important because dry and wet deposition components contribute external sources of N to ecosystems, and the role of dry NH_3 and wet NH_4^+ deposition pathways continue to be prominent in federally protected environments.

Efforts are in place to protect alpine environments using empirical critical loads, but federal emission regulations do not necessarily adhere to these suggested deposition thresholds because not all precursory trace gas emissions are regulated (e.g., NH_3). With decreases in national NO_3^- deposition trends, there has been an expectation that surface water NO_3^- concentrations in pristine alpine environments should also be in decline. However, this is not the case and elevated concentrations of NO_3^- persist in alpine watershed surface waters in ROMO (e.g., Loch Vale). It has been suggested that these concentrations are connected to changes in

hydrology, glacial thaw and/or increasing NH_4^+ deposition (Baron et al., 2009; Barnes et al., 2014); the latter could fuel microbial conversion (i.e., nitrification) of atmospheric NH_4^+ to form NO_3^- . If surface water concentrations still reflect high concentrations under revised federal emission standards, how can surface water concentrations be improved/reduced? To refine our understanding of what drives concentration/deposition trends in these high elevation environments, we take a regional approach to look at this in more detail.

Atmospheric N is determined by: emissions, chemistry, atmospheric transport, and deposition. In mountain ecosystems, the elevational terrain introduces complex gradients for air parcels to be lofted and gas-phase and particulates to be washed out or dry-deposited. In ROMO, it has been observed that large supplies of N are transported to high elevation areas via easterly upslope flow events during spring, summer and even fall precipitation events (Beem et al., 2010; Benedict et al., 2013a-b; Gebhart et al., 2011). These upslope events occur when low pressure centers push moist air across the eastern plains and urban areas into the mountains causing orographic uplift and precipitation with gains in elevation (Wetherbee et al., 2019). While predominant wind directions are from the west, it has been suggested that up to 50% of yearly wet N deposition occurs during easterly upslope events (specifically in April and July) from localized emission sources (e.g., NH_3) (Beem et al., 2010, Benedict et al., 2013; Gebhart et al., 2011).

It remains to be seen whether trends in atmospheric inputs of annual and seasonal changes in DIN concentration in these high elevation areas are driven by shifts in emission sources alone or changes in transport over long temporal periods. If there is no change in transport, then concentration/deposition trends are presumably related to emissions, chemistry, deposition or some combination of these. On the other hand, if there is a change in transport,

then emission sources are likely not the sole driver of changes in reactive N being delivered to these remote environments. To our knowledge, long-term trends in transport have not been thoroughly investigated to address this question. We investigate this by looking at long-term trends in transport at one alpine site as a case-study in the Colorado Front Range. First, we focus on understanding long-term trends in inorganic NO_3^- and NH_4^+ deposition across alpine sites of the Western U.S. to address whether regional trends in DIN correspond with national-scale trends observed. Then, we present trends in atmospheric transport and corresponding trends in concentration at annual and seasonal scales in an attempt to tease apart the importance of transport changes relative to these long-term deposition trends.

2. Methods and Data Analysis

2.1 National Atmospheric Deposition Program Time Series

Since 1978 the National Atmospheric Deposition Program (NADP) has been monitoring precipitation amount and chemistry across the United States and currently consists of 263 stations. Wet precipitation samples are collected using standardized 3.5-gallon buckets paired with a precipitation sensor to trigger the beginning and ending of sampling. Precipitation is collected weekly, measured for volume and analyzed for a suite of chemical compounds (e.g., NO_3^- , NH_4^+ , SO_4^{2-} , Ca^{2+} , Mg^{2+} , Cl^- , Na^+ , K^+ , pH) at the Wisconsin State Laboratory of Hygiene, University of Wisconsin (nadp.slh.wisc.edu). Data are presented as measures of concentration and deposition, where concentration represents mass of solute per unit volume of water (e.g., mg/L) and deposition reflects mass of pollutants per unit area per unit time (e.g., kilograms N/ha/yr) (nadp.slh.wisc.edu). Concentrations are presented here as milligrams per liter of water (mg/L). Annual and seasonal averages for each measure are volume-weighted and available

publicly (nadp.slh.wisc.edu). Seasonal data are defined as: winter (DJF), spring (MAM), summer (JJA) and fall (SON).

To understand trends in atmospheric inputs, linear regression via R version 3.3.3. was used to assess whether annual inorganic N deposition and concentration trends during two time periods were significant at all 54 NADP/NTN sites from the western United States with collection records extending from 1990 through 2018. Data were analyzed in two separate time periods, 1990-2000 and 2001-2018 to distinguish any significant trends in the average annual NO_3^- and NH_4^+ deposition or concentration across all 54 sites before and after the year 2000. Trends in mean annual concentration and deposition for NO_3^- and NH_4^+ were assessed by site using the slope from each regression analysis to delineate the trend and whether it was statistically significant (defined as a p-value ≤ 0.05). This analysis was also performed for alpine sites only, where alpine sites were defined by as an elevation above 2500 meters (based on Williams and Tonnessen, 2000). Twelve sites from Wyoming and Colorado met the alpine criterion. Linear regression was also used to assess NADP annual and seasonally averaged concentration data at a selected alpine site in Colorado (CO-02, Niwot Saddle). All data were accessed from the publicly available NADP database (<http://nadp.slh.wisc.edu/data/sites/list/?net=NTN>).

2.2 HYSPLIT Airmass back-trajectory and clustering analysis

To determine the role of long-term trends in airmass transport in inorganic N delivery to an alpine NADP site within the Colorado Front Range, airmass back-trajectories were modeled from 1997 to 2016 at Niwot Saddle (40°03'16.9" N, 105°35'20.8" W, elevation: 3520 meters) using NOAA's Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) Model (Draxler and Hess, 1998; Stein et al., 2015). Back-trajectories were calculated using NOAA's

National Centers for Environmental Prediction (NCEP) Eta Data Assimilation System (EDAS) gridded meteorological data, which is provided by NOAA's Air Resources Laboratory (NOAA-ARL). The EDAS meteorology data used for this analysis extends back to 1997 (Black, 1994). However, we note a change in the resolution of the grid size during this time period, where data between 1997-2003 had a resolution of 40 km compared to 80 km for 2004-2016 (Black, 1994; Stein, 2015). Because this change in resolution results in different average elevations, starting heights above ground level (AGL) of 1000 and 1500 meters were used for the 40 km and 80 km datasets, respectively. Each dataset assumes the respective ground level is 500 and 1000 meters below the actual level, therefore to accurately model the elevation of the receptor site (~3500 meters), these selected starting heights (1000 and 1500 meters) reflect a real starting height of 500 meters AGL for both grid sizes. Back-trajectories were calculated every 6 hours (i.e., 0, 6, 12, and 18 UTC) for a total of 4 trajectories per day for 4 days (96 hours) over the entire 20-year period (1997-2016). This length of time for each back-trajectory (96 hours) was selected to account for/capture the atmospheric lifetimes of NH_4^+ (1/2 day to 3.2 days, Asman et al., 1998) and NO_3^- (1 to several days, Levy et al., 1999). Three to four-day back trajectories adequately represent regional-scale transport within acceptable limits of uncertainty for the spatial resolution of the gridded meteorology dataset, in which uncertainty in computed back-trajectories increases with distance from the starting location beyond 72 hours (Kahl and Samson, 1986; Gratz et al., 2013)

Using the generated back-trajectories, a statistical clustering analysis was completed using five 4-year time periods from this dataset including: 1997-2000, 2001-2004, 2005-2008, 2009-2012, and 2013-2016 via HYSPLIT's Clustering Algorithm. These temporal groups were created to ensure that a systematic method of clustering occurred, such that more meaningful

long-term trends would be apparent from multi-year clustering compared to clustering single years of back-trajectory data. In addition, HYSPLIT could more efficiently complete the computational analysis with this large amount of data (~29,000 back trajectory files) if it was divided up into smaller classified groups. Clusters were determined based on 72-hour clustering of airmass groups that maximized the total spatial variance (TSV) using each back-trajectory and was completed for each 4-year period where the differences between trajectories within clusters are minimized and the distance between clusters are maximized (Stunder, 1996; Borge et al., 2007). Provided this temporal setting, files below a particular size/shorter than 72 hours were removed prior to the clustering analysis. Airmass clustering trends were then compared between each 4-year period over the longer 20-year data set, as well as within runs, to assess differences or similarities in the quantity of airmasses that followed a particular pathway. To assess the relative importance of transport pathways over time, the total proportional contribution of each pathway (TPPC) was calculated by dividing the total trajectory number assigned to a specified pathway by the total number of trajectories clustered during each 4-year period clustered (Eqn 1). The annual proportional contribution (APPC) within a given pathway was also determined per 4-year period using Equation 2. Trajectories that fell into the 0th cluster were not included in these calculations (i.e., total trajectories = total trajectories assigned to a non-0 cluster). Equations 1 and 2 were used for each 4-year (annual, seasonal) and all 20-year (monthly) clustering groups:

$$(1) \text{ TPPC (\%)} = \text{trajectories pathway}^{-1} \text{ 4-yr-group}^{-1} / \text{total trajectories 4-yr-group}^{-1}$$

$$(2) \text{ APPC (\%)} = \text{trajectories yr}^{-1} \text{ pathway}^{-1} \text{ 4-yr-group}^{-1} / \text{total trajectories 4-yr-group}^{-1}$$

The same clustering analysis was conducted at a seasonal scale, where trajectories were assigned to the same 4-yr periods according to 3-month seasonal groups where winter = DJF,

spring = MAM, summer = JJA, and fall = SON. Equation 1 was also used to calculate the seasonal contribution of specific pathways by 4-year clustering groups (includes 3 months of trajectories per year), and is referred to as the Seasonal Proportional Pathway Contribution (SPPC). These results were used to compare with and assess whether seasonal changes in concentration data correspond with trends in transport based on the clustering analysis. Seasonal concentration data was qualitatively compared in the same synchronous 4-yr periods for easy comparison with the transport results. While concentration trends may differ among alpine sites (similar in elevation, but far apart), transport trends are not anticipated to differ greatly, therefore we use CO-02 as a representative case study for the region. Lastly, clustering analyses containing the entire 20-yr period of trajectories for a few individual months during spring and summer (e.g., April, May, July) were conducted in an attempt to capture easterly upslope transport events in this analysis that are highlighted in previous work in the region (Beem et al., 2010; Benedict et al., 2013, etc). Equations 1 and 2 were used to calculate proportional changes in monthly transport (April and July) using all 20 years of trajectory data in a single clustering analysis.

3. Results

3.1 NADP trends at alpine sites of the Western US over time

Across the 54 western sites, trends in NO_3^- and NH_4^+ deposition and concentration were not the same everywhere, especially prior to 2000 when a smaller percentage of sites showed significant trends (Table 1). This contrasts with observations post-2000 where 56 and 66% of the sites revealed significant declines in NO_3^- (deposition and concentration, respectively) and increases in NH_4^+ (66 and 38% of sites resembling this trend, respectively) (Table 1).

Trends in NO_3^- deposition at alpine sites were not uniform compared to observed western trends before 2000 (Table 1) or national trends portrayed in NADP deposition maps (NADP,

2019). While most sites showed an increasing trend, only 27% of the high alpine sites showed a significant increase in NO_3^- deposition before 2000, while 92% showed a significant decrease after 2000, which is consistent with regional observations (Table 1). No specific trend or statistically significant relationship was observed in NH_4^+ deposition across alpine sites before 2000 (Table 1). In contrast, more than half of the alpine sites (83%) showed a significant increase in NH_4^+ deposition between 2001-2018 (Table 1). Between 1990-2000, only a small proportion (17%) of alpine sites showed significant increases in NO_3^- concentration while all sites significantly decreased (100%) from 2001 onward (Table 1, Figure 1). In contrast, a small proportion of alpine sites experienced a significant decrease (17%) and increase (25%) in NH_4^+ concentration before and after 2000, respectively (Table 1, Figure 1). See Table 1 for complete regression analysis results.

		1990-2000		2001-2018	
		Incr. (% sig)	Decr. (% sig)	Incr. (% sig)	Decr. (% sig)
West n=54	NO_3^- Dep	48% (12%)	52% (0%)	24% (8%)	76% (56%)
	NH_4^+ Dep	32% (18%)	69% (5%)	87% (66%)	13% (29%)
	NO_3^- Con	50% (22%)	50% (22%)	0% (0%)	100% (67%)
	NH_4^+ Con	33% (11%)	67% (28%)	83% (38%)	17% (11%)
Alpine n=12	NO_3^- Dep	92% (27%)	8.3% (0%)	0% (0%)	100% (92%)
	NH_4^+ Dep	58% (0%)	42% (0%)	83% (70%)	17% (50%)
	NO_3^- Con	75% (17%)	25% (0%)	0% (0%)	100% (100%)
	NH_4^+ Con	33% (0%)	67% (17%)	92% (25%)	8.3% (0%)

Table 1: NADP NO_3^- and NH_4^+ deposition and concentration trends between 1990-2000 and 2001-2018. Trends are identified by the percentage of alpine or western sites that show an increasing or decreasing trend in deposition/concentration, followed by the percentage of sites that show a significant positive or negative trend (where significance is defined as $p \leq 0.05$). The trends that are significant are in bold.

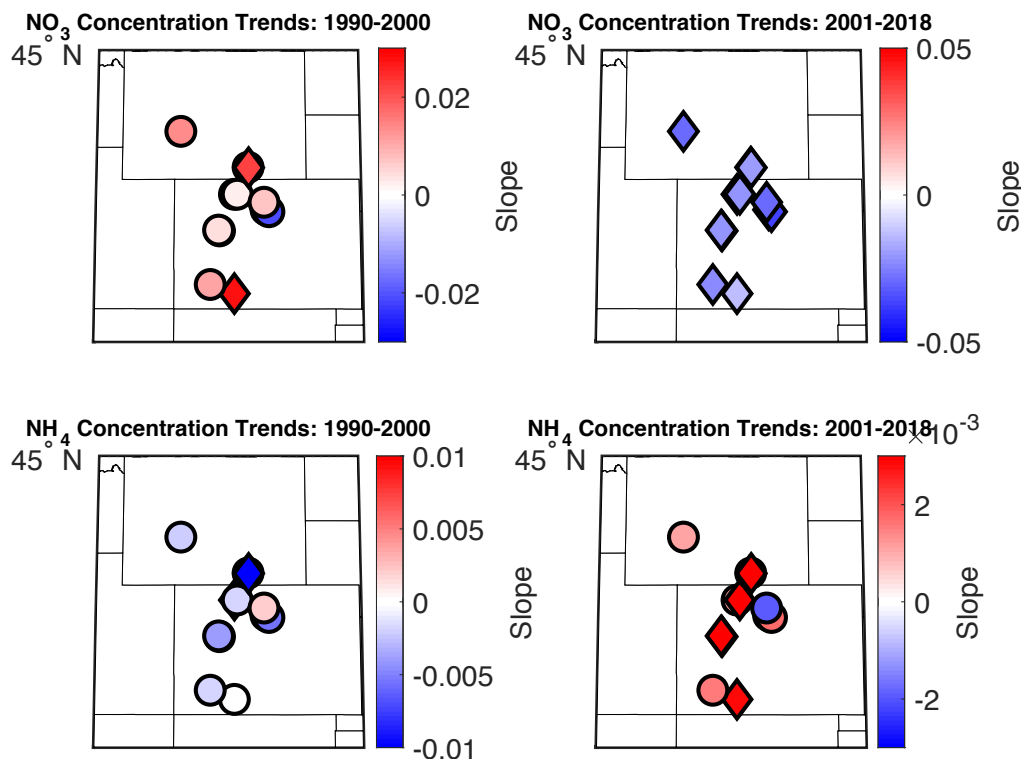


Figure 1: NADP annual concentration trends for NO_3^- and NH_4^+ across 12 high alpine network stations between 1990-2000 and 2001-2018 in the western United States. High alpine is defined as a station located above 2500 meters in elevation. Symbols reflect a significant (diamond) or insignificant (circles) trend. Statistical significance is defined as having a p-value ≤ 0.05 . Slope trends are represented by the color scale, where positive and negative trends are reflected by red and blue, respectively.

While trends across alpine sites were not all uniform, mean annual and seasonal concentration trends for NO_3^- and NH_4^+ at one alpine site, Niwot Saddle, generally demonstrated the same trend as observed nationally (i.e., declining NO_3^- , increasing/unchanging NH_4^+). Average annual concentrations at Niwot Saddle showed a significant decreasing trend for NO_3^- ($R^2 = 0.1494$, p-value = 0) and a gradually increasing, but insignificant trend for NH_4^+ ($R^2 = 0.0114$, p-value > 0.3 ; Figure 2). Seasonal mean concentrations for NO_3^- at this location revealed declining trends in all seasons, but the highest observed concentrations occurred in spring and summer, which were also the most variable compared to fall and winter (Figure 3a). In contrast, seasonal mean NH_4^+ concentrations show gradually increasing/unchanging trends over the same

time interval (1997-2016) across all seasons (Figure 3b). Similarly, the highest variability in mean concentrations was observed in spring and summer and the lowest variability was in fall followed by winter (Figure 3b).

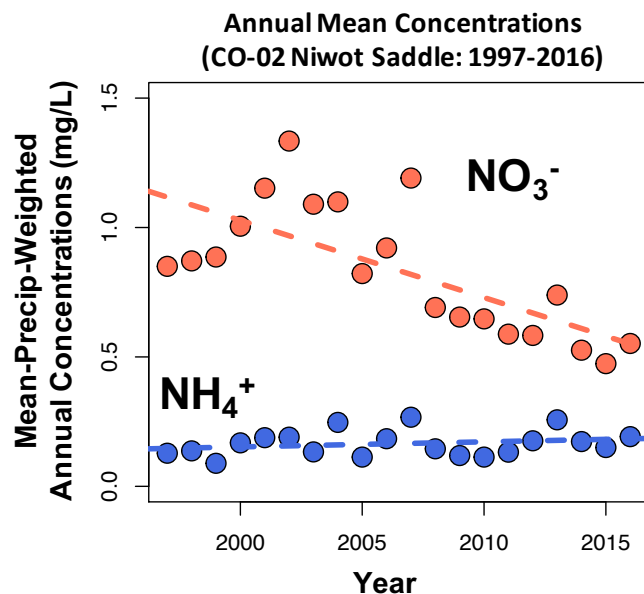


Figure 2: Mean annual volume-weighted concentration for nitrate and ammonium (mg/L). NH_4^+ concentration ($R^2 = 0.0114$, $p\text{-value} > 0.3$); NO_3^- ($R^2 = 0.1494$, $p\text{-value} = 0$).

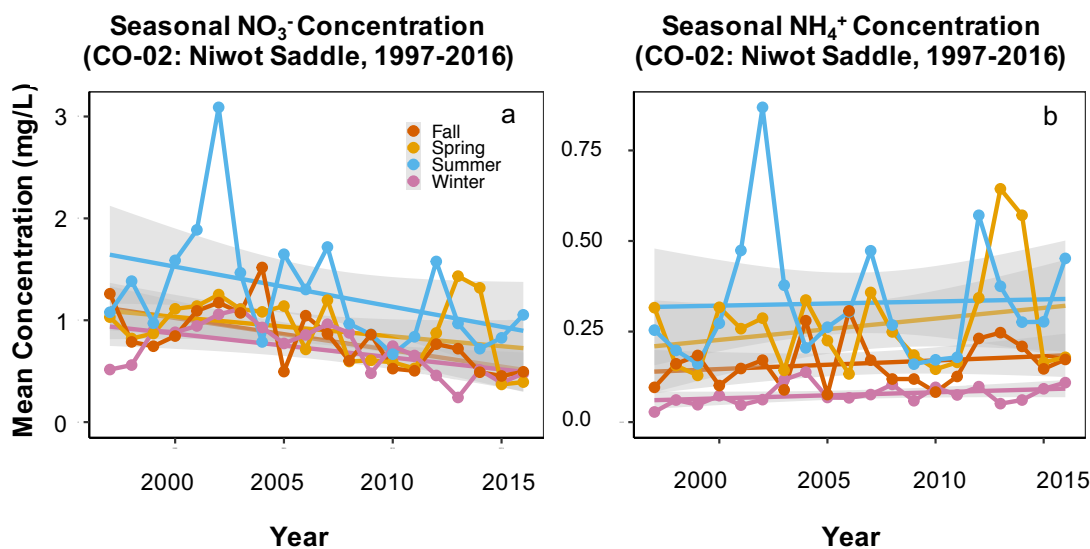


Figure 3: Seasonal trends in (a) nitrate and (b) ammonium concentration (mg/L) from 1997-2016, where seasons are color-coded (fall = red, spring = orange, summer= blue, and winter = pink). Gray envelope for each linear model is 95% confidence interval.

3.2 HYSPLIT Back-Trajectory and Clustering Analysis

Under the 72-hour analysis, all five 4-yr periods: 1997-2000, 2001-2004, 2005-2008, 2009-2012, and 2013-2016 showed similarities in westerly transport trends. There were 3 transport pathways over the course of the analytical period: 1) a dominant transport pathway that originated from the Pacific Northwest/Pacific Ocean, 2) another that originated from the southwestern United States, specifically Arizona and 3) a more regional short-range pathway, specifically Utah and western Colorado (Figures 4, S1). These pathways are referred to as PNW, SW and Local throughout the rest of the text. The proportional contribution (APPC) to annual transport trends revealed that the contribution of transport from this regional, short-range pathway has increased in importance over time by ~10% ($R^2 = 0.733$, $p = 1.486 \times 10^{-6}$) relative to the PNW and SW pathways which have significantly decreased over time by ~5% ($R^2 = 0.5073$, 0.4215 and p -values = 0 for PNW and SW, respectively; Figure 5). A dramatic uptick in the contribution (TPPC) of this Local pathway occurred around 2005, such that it comprised more than 50% of the transport arriving at this high elevation site from 2005 to 2016 and was consistent with a decrease in the PNW and SW pathways (Figure 4, S1, Table 2).

Years Clustered	Local (% of transport)	PNW (% of transport)	SW (% of transport)
1997-2000	25	36	39
2001-2004	31	40	29
2005-2008	56	21	23
2009-2012	53	15	31
2013-2016	58	21	21

Table 2: Percentage of trajectories that compose primary transport pathways at annual, 4-year clustering time scales (TPPC calculations).

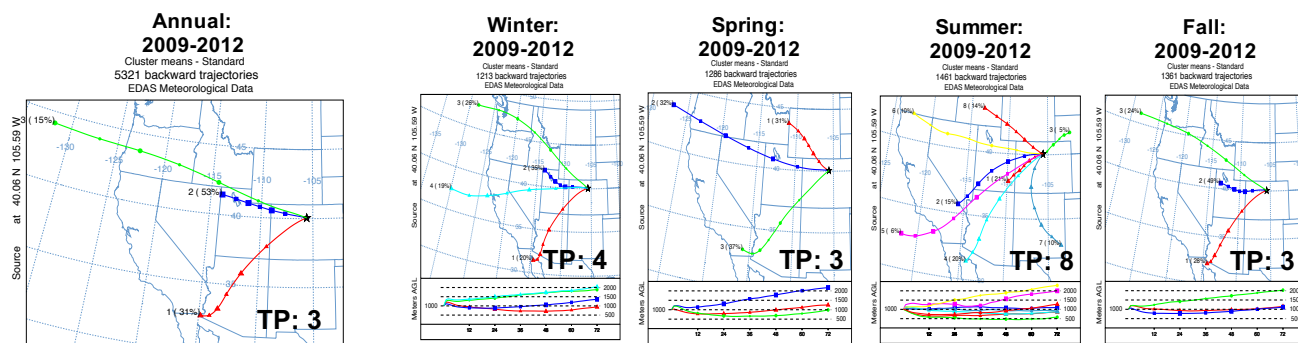


Figure 4: Example of annual and seasonal breakdown for transport clustering results in 2009-2012. Transport pathways (TP) resulting from the clustering analysis are identified in each map showing that at annual scales, there are 3 consistent pathways, but at a seasonal scale transport becomes more variable. Note that the color of the pathways is arbitrarily assigned by HYSPLIT and not indicative of consistent pathway identification between temporal analyses. See supplemental Figure S1 for transport maps for all 20 years.

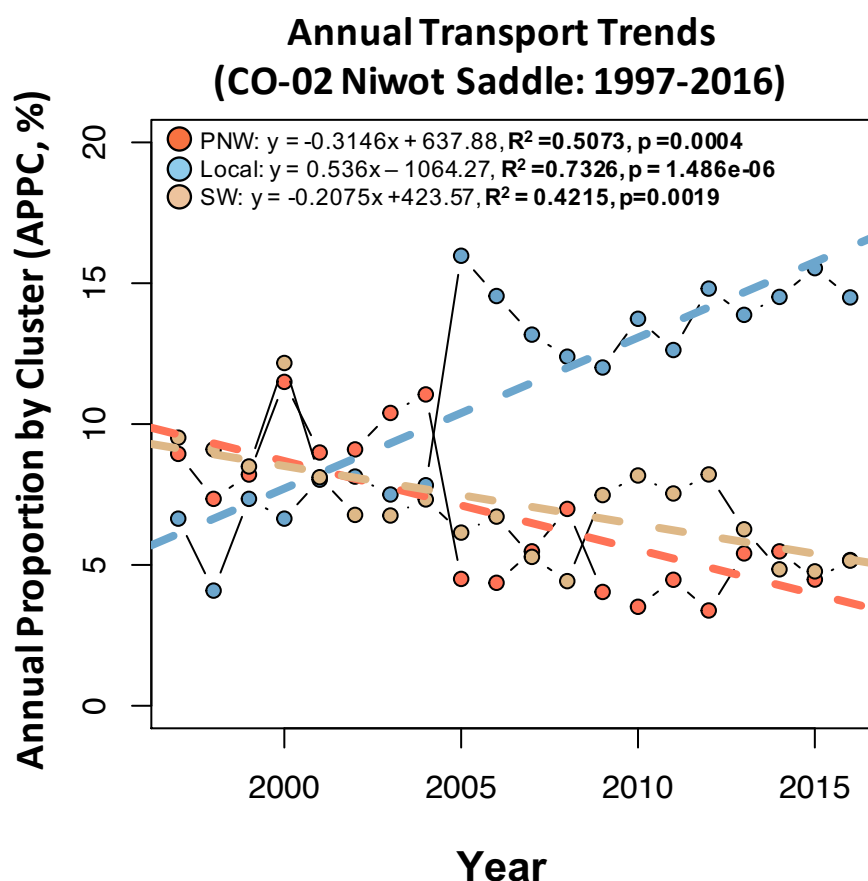


Figure 5: Annual (APPC) trends in atmospheric transport based on back-trajectory clustering analysis at Niwot Saddle (NADP Site CO-02). Local transport shows a significant increasing trend, while PNW and SW transport relationships show significant decreasing trends ($p=0$).

On seasonal time scales, the clustering analysis resulted in more variable transport during spring and summer seasons (3 – 8 pathways), while two pathways were observed consistently for winter and fall (Table 3). This was evident for all 4-yr clustering periods except 2009-2012, when more variable transport was observed across all seasons (Table 3). Surprisingly, easterly transport defining upslope events does not stand out as a major feature in the transport analysis on annual or seasonal time-scales. To investigate this further and verify that the changes seen over the 4-year periods was not somehow coincidental, two months (April and July) were clustered in two discrete time periods (1997-2007 and 2008-2016) to assess whether the same variability in transport was captured that was observed starting in 2009-2012 (Table 3). Results reflected 3 transport pathways in April and July before 2007 and 4 and 5 pathways for April and July, respectively after 2008 (data not shown). Further exploration of this seasonal variability at monthly time scales (e.g., April, May and July) demonstrated that more variable transport occurred in April than in May or July where transport pathways reaching the receptor site were 6 (April) and 3 (May and July), over the entire 20-yr period (Figure 6, May not shown). This exercise demonstrated that easterly transport was detectable at monthly resolutions over this time period in April and July from a northeastern (South Dakota) and southeastern (within Colorado) origin (Figure 6). In contrast, easterly transport was not detected in May (not shown). Of the 6 transport pathways observed in April during this period, transport from the SW (i.e., Arizona) comprised the largest proportion (33%) relative to other westerly pathways (California (8%), North-Canada (10%), Pacific/Northwest (17%), Northeast (South Dakota, 12%) and Local (20%)) (Figure 6a). Transport from the SW comprised the largest proportion of transport relative to the Local and PNW pathways, which demonstrated the greatest change (i.e., significant increase over time) and the next two largest contributions to the transport composition at Niwot

Saddle (20% and 17% of April composition, respectively (Figures 6a, 7)). Similarly, transport from the SW comprised the largest proportion in July (38%) followed by the PNW (32%) and Southeast Colorado (30%) (Figure 6b). Of these three main transport pathways, the annual proportional contribution (APPC) of transport from Southeast Colorado significantly increased over the 20-year period by ~5% ($p=0.02$, Figure 7).

Years Clustered	Winter	Spring	Summer	Fall
1997-2000	2	5	3	2
2001-2004	2	4	3	2
2005-2008	2	2	5	2
2009-2012	4	3	8	3
2013-2016	2	5	4	2

Table 3: Seasonal back-trajectory clustering results from 1997-2016.

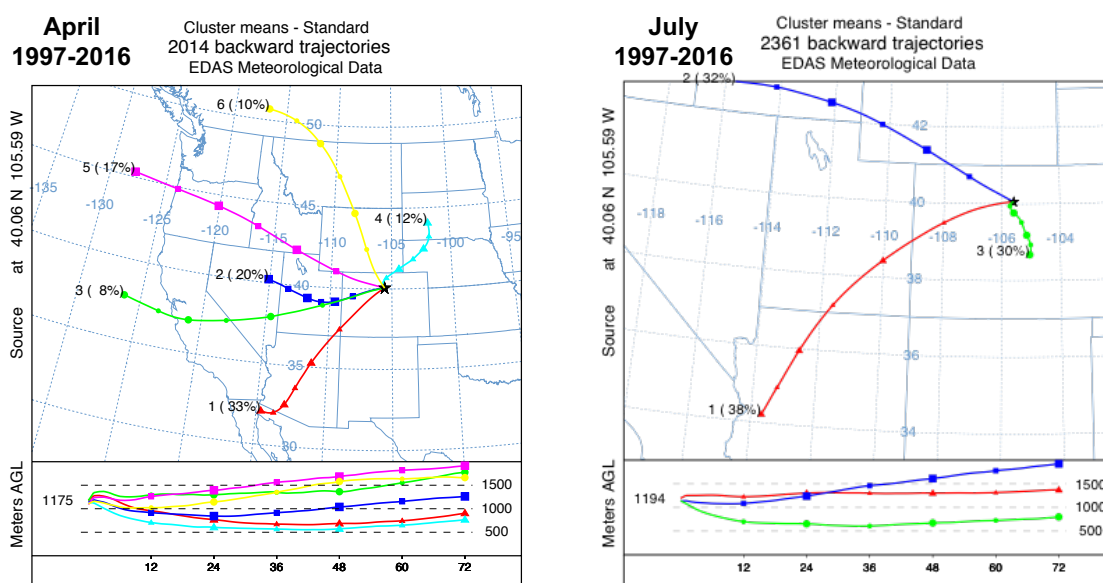


Figure 6: Spring and summer monthly clustering results comparison of April and July (TPPC) from 1997-2016.

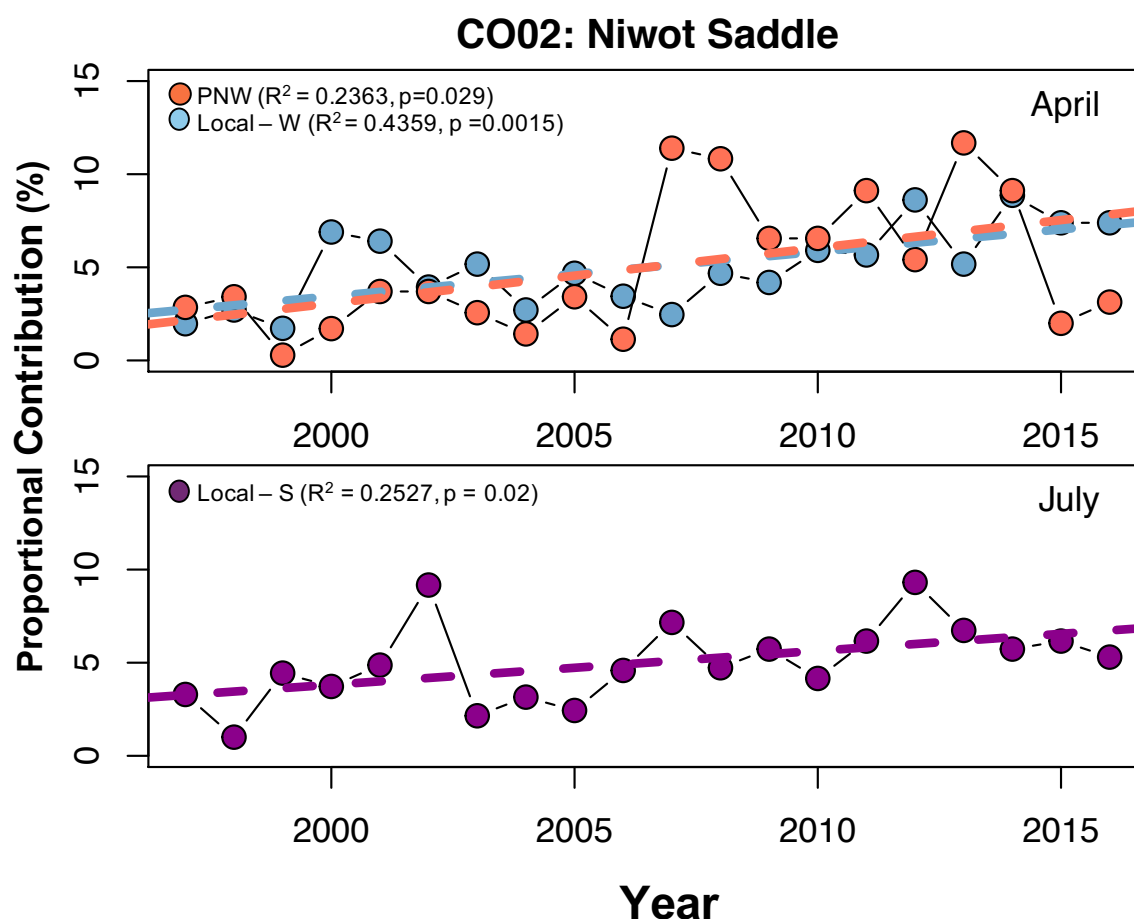


Figure 7: Annual proportional change (APPC) in April and July transport from 1997-2016 for pathways that show significant trends. Of the six transport pathways in April transport, only Pacific-Northwest (PNW) and Short Range (Local) transport show significant trends over time (p -value <0.05). Of the three pathways in July, only short-range transport from Southeast Colorado (Local-S) shows a significant trend ($p=0.02$). All other transport relationships are insignificant, thus not shown.

4. Discussion

The observed change in long-term inorganic N deposition trends has largely been attributed to air quality regulations (NO_x only) and source changes (Li et al., 2016; Fenn et al., 2018), but the role of transport has not been examined as extensively for long-term records of deposition chemistry. In this study, we first examine whether concentration trends at high elevation NADP sites match national trends over a 30-year period, then evaluate whether annual and seasonal concentration trends and transport pathways over a 20-year period provide insight

regarding the role of long-term shifts in transport and the observed concentration changes using one high elevation site in Colorado as a case study.

4.1 Long-term NADP Trends

Over the past 30 years, trends in NO_3^- and NH_4^+ concentration at alpine sites more closely reflect national observations, but this does not appear to be the case across the entire western region (Figure 1; Table 1). For example, NH_4^+ has remained constant/unchanged over time in some locations further west than Colorado, while a general increasing trend occurs at sites along the continental divide – many of which show significant trends (not shown). While we do not delve into this further, it is interesting that trends across this region are not more uniformly consistent with national trends while the opposite appears true for high elevation sites in Wyoming and Colorado (i.e., alpine, Figure 1).

4.2 Local westerly transport has increased in importance over time

Since most alpine sites follow national observations, we delve into the annual and seasonal trends in inorganic N concentrations and transport from 1997-2016 to determine whether transport changes align with concentration trends at Niwot Saddle. Annual NO_3^- concentrations have significantly declined, while NH_4^+ has gradually increased/remained constant from 1997-2016 at Niwot Saddle (Figure 2) – this trend is consistent with inorganic N observations at another nearby alpine site in ROMO, though the authors suggest that annual and seasonal deposition may be biased estimates due to precipitation amount dependencies on deposition calculations and missing data (Schichtel et al., 2019). These patterns also align with a significant increase in short-range, localized westerly transport (Local) and declines in transport from the Pacific/Pacific Northwest (PNW) and the Southwest (SW) (Table 2; Figures 5, S1),

which make up 47%, 25% and 28% of the transport composition over the 20-yr period.

Unsurprisingly, the predominant westerly wind direction dictates the majority of transport being delivered and distributed to this receptor site at annual scales.

4.3 Seasonal differences in transport and wet inorganic nitrogen concentrations

Patterns in inorganic N concentrations can be related to timing of specific activities responsible for emissions, especially NH_3 (e.g., agricultural fertilizer application, influence of warming temperatures on volatilization) and are important to consider when assessing concentration and transport trends over this analytical period. In an attempt to capture transport changes that might correspond with seasonal differences in concentration, we fine-tune our analysis to isolate seasons and assess whether distinct trends in seasonal transport, including easterly flow, occurred over this time frame.

More variable transport occurs during spring and summer seasons, which aligns with when concentrations of both NO_3^- and NH_4^+ tend to be highest and exhibit the most variability (Table 3, Figures 3-4, 6-7). Specific months within these seasons exhibit more (April) and less (May, July) variable transport over the entire 20-year period (Figure 6). Though merely qualitative, there are some similar features observed in the concentration and transport time series. For example, spring NO_3^- and NH_4^+ concentrations peak in 2007 and 2013 followed by a descent (2014-2016), which are comparable to higher contributions from the PNW pathway during those years in April (Figures 3, 7). In contrast, the proportional contribution of the Local pathway appears to be less important during these periods for April (Figure 6). Similarly, high summer NO_3^- and NH_4^+ concentrations in 2002, 2007, and 2012 correspond with peaks in July transport from southeast Colorado (Local-S; Figure 3,7). Easterly transport is detectable at monthly scales in April (originating from South Dakota) and July (originating from southeastern

Colorado) (Figure 6) corroborating previous observations (Beem et al., 2010; Benedict et al., 2013). However, in both cases these easterly flow-paths comprise some of/the smallest proportional contribution (TPPC) in transport to this site over the 20-year time period (April: 12% and July: 30%, Figure 6). Otherwise easterly transport events were not detected at seasonal scales (Figures 4, S1). This is likely a function of the resolution of the meteorology dataset used and the time-scale of analysis tested (3-months in a season), as the clustered modes of transport in seasonal analyses must not maximize the total spatial variance to yield easterly flow as a significant cluster. Perhaps the proportional contribution to transport would yield different results using a higher resolution meteorology dataset. Interestingly, previous work using back-trajectory analysis for reactive N source apportionment in the region found that EDAS is less sensitive/effective at capturing the diurnal circulation patterns in this complex terrain compared to MM5 as a function of slower wind speeds (e.g., easterly upslope events; Gebhart et al., 2011). Overall the observed trends in seasonal westerly transport align with previous back-trajectory and chemical tracer results in the region (Gebhart et al., 2011; Gebhart et al., 2014; Benedict et al., 2018) and we are able to capture some easterly flow in specific spring and summer months, but the fact that we observe a long-term shift in local transport from the west is interesting and worth noting.

5. Conclusions

Results from this study suggest that changes in atmospheric transport over time are apparent and likely contribute to some of the differences in the observed DIN concentration trends. Potential sources that could explain the seasonality or that are encountered along major transport pathways observed in this study are discussed below.

The dominant role of dry NH_3 and wet NH_4^+ compared to other deposition pathways

previously observed in ROMO speaks to the consequential impact of unregulated NH_3 emissions (Beem et al., 2010; Benedict et al., 2013a-b; Benedict et al., 2018) presumably from agricultural activities and urban vehicles (Fenn et al., 2018; Li et al., 2016). If vehicles are an important source of NH_3 , you might expect the concentration signal to be somewhat constant in seasons (fall/winter) without large changes in temperature (summer) or applications of NH_3 -rich fertilizers (spring). In fact, we observe steady wintertime NH_4^+ concentrations over time, which consistently reflect the lowest seasonal concentrations (Figure 3b). This may yield preliminary evidence that wintertime NH_4^+ concentrations correspond to vehicle emissions relative to other seasons. However, vehicles as an NH_3 source cannot be determined from concentration alone. If the $\text{NH}_3:\text{NO}_x$ ratio is not 1:1 during catalytic conversion of NO_x , then the reduction process is inefficient resulting in under-reduction leading to NO_x emissions or an over-reduction leading to NH_3 slip (Fenn et al., 2018). Isotopic work using $\delta^{15}\text{N}-\text{NO}_x$ and $\delta^{15}\text{N}-\text{NH}_3$ indicates that vehicle fleet composition is important based on differences between urban fleet emissions that use three-way catalytic conversion relative to diesel trucks which use selective catalytic reduction (Miller et al., 2017, Walters et al., in review). There is great potential for the seasonal role of source changes in this region to be resolved with further developments of $\delta^{15}\text{N}$ isotopic source constraints on NH_3 and NO_x to constrain source contributions, as well as, the role of chemistry and resulting impact on deposition patterns.

The role of regional/local short-range transport has become more important over time, which also aligns with shifts in dissolved inorganic N (DIN) composition (decrease in NO_3^- , increase in NH_4^+) and when variable transport during spring and summer is observed (e.g., higher and more variable NH_4^+ in summer). While prior work demonstrates that important contributions of reduced N are primarily sourced from in-state/easterly sources (Beem et al.,

2010, Benedict et al., 2013), estimates using Transport Mass Balance models (TrMB) and hybrid deterministic-receptor approaches suggest that ~50% of the NH₃ emissions are sourced outside of the state and to the west, while the other 50% comes from within the state though source apportionment remains difficult (Malm et al., 2013; Gebhart et al., 2014). It is extremely challenging to distinguish source contributions using concentration and transport data alone and really requires more of a chemical tracer/isotopic approach to conduct source apportionment since sources are variable and transport distance will vary depending on phase (gas vs particle). It is important to consider contributions from urban NH₃ sources and the increasing impact of wildfires in the western U.S. (Fenn et al., 2018; Bray et al., 2016) to deposition trends along non-easterly pathways. Also, an unexpected slowdown in NO_x due to suspected increasing contributions from industrial, small-scale industrial “area”, and off-road mobile sources with simultaneous decreases in gasoline vehicles and slow declines in diesel NO_x has also taken place in areas such as the southwestern U.S. (Jiang et al., 2018). Consequently, incoming sources of oxidized N may become important since localized westerly transport seems to have increased over time (observed in this study).

The National Emission Inventory database allows us to qualitatively assess large emission sources along the primary transport pathways observed in this study. NO_x sources from oil and gas development, as well as, coal-fired power plants are located along the transport pathway from the SW (Rodriguez et al., 2009; Li et al., 2016). Additionally, the Localized westerly transport coming from northern Utah (near Great Salt Lake) is close to NO_x emitters including the Salt Lake City Airport, a power plant in Magna, as well as copper and nickel ore mining operations near Bingham Canyon (NEI, 2017). Large sources of NO_x in Oregon and Washington located within range of the PNW transport pathway include a power plant in

Centralia (WA), metropolitan airports (Seattle and Portland), petroleum refineries, and mills associated with the lumber industry (saw, paper and pulp mills) (NEI, 2017). There are likely urban NH_3 sources (i.e., vehicles) that play an important role along these pathways as well (Fenn et al., 2018). These results suggest that the increasing significance of localized, short-range transport of source emissions west of this high elevation site may require additional regulatory measures of NO_x and NH_3 emission sources in western Colorado and northern Utah moving forward.

While this is beyond the scope of this work, recent studies have refined the $\delta^{15}\text{N}$ - NO_x source signatures for vehicles, soils and biomass burning using standardized methods (Miller et al., 2017; Miller et al., 2018; Fibiger and Hastings, 2016; Chai et al., 2019), which are understood to be conserved from emission as NO_x to deposition as NO_3^- . Insights can also be gained from oxygen isotopes of NO_3^- ($\delta^{18}\text{O}$ - NO_3^- and $\Delta^{17}\text{O}$ - NO_3^-) in wet deposition regarding seasonal changes in oxidation chemistry (Michalski et al., 2012 and references therein). Similarly, new methods for $\delta^{15}\text{N}$ - NH_3 characterization (Walters and Hastings, 2018), insights regarding fractionation effects during gas-particle conversion (Walters et al., 2019), and constraints on the $\delta^{15}\text{N}$ - NH_3 of vehicle fleets (Walters et al., in review) offer exciting opportunities for improving our understanding of NH_3 source and chemistry dynamics. Collectively, these tools may help completely constrain reactive N budgets in the future and improve management of natural resources.

As more studies highlight the growing presence of NH_3 source emissions and mounting threats to critical loads in sensitive ecosystems, it is clear that there is more incentive to regulate emissions of NH_3 because the impact of inorganic N deposition (wet and dry) continues to be problematic. Significant declines in observed wet deposition of NO_3^- in regions of the U.S.

where major emission reductions of NO_x have taken place corroborate the fact that federally mandated emission standards can be successful. While the role of easterly sources of inorganic N should be monitored, the increasing prevalence of local, westerly transport indicates that additional regulatory measures of NO_x and NH₃ emission sources in western Colorado and northern Utah may be required moving forward if sensitive ecosystems, such as high elevation mountain watersheds, are to be effectively monitored and protected.

6. Acknowledgements

We thank our funding sources from Colorado College, Brown University and Institute at Brown for Environment and Society and members of the Hastings Lab for useful discussions of this work. There are no competing interests.

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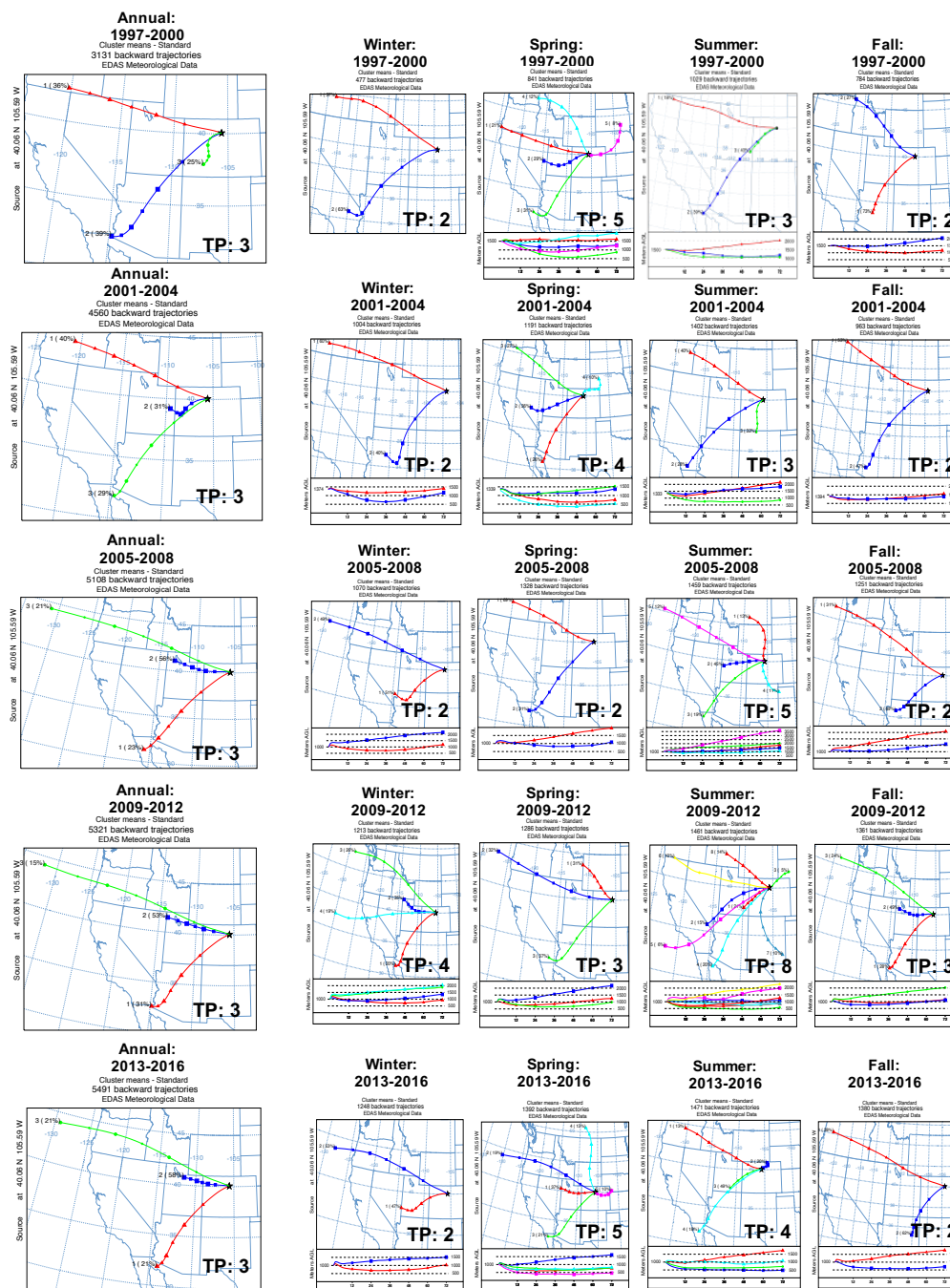


Figure S1: Annual and seasonal breakdown by 4-year clustering periods for 1997-2000, 2001-2004, 2005-2008, 2009-2012, and 2013-2016. Transport pathways (TP) resulting from the clustering analysis are identified in each map showing that at annual scales, there are 3 consistent pathways, but at a seasonal scale transport becomes more variable. Note that the color of the pathways is arbitrarily assigned by HYSPLIT and not indicative of consistent pathway identification between temporal analyses.

Chapter Five

Quantifying atmospheric nitrogen deposition to alpine ecosystems of the Colorado Front Range

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In Prep for *Environmental Science and Technology*

Abstract

Nitrate concentrations in high elevation lakes of the Colorado Front Range remain elevated despite declining trends in atmospherically deposited nitrate since 2000. The current source of this elevated nitrate in surface waters remains elusive and is complicated by additional nitrogen sources including atmospheric ammonium deposition and glacial inputs. We asked: is atmospheric deposition still fueling high lake nitrate concentrations? We present the complete isotopic composition of nitrate ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$) in a suite of sample types collected during the summers of 2017-2018 from two alpine ecosystems in an attempt to constrain the provenance of elevated nitrate in surface waters over the course of the summer thaw season. Results indicate that alpine lakes are sustained by a consistent contribution from uncycled atmospheric nitrate throughout the summer season (11-20%), however the dominant supply is microbially produced (as high as 89% in late summer, possibly from nitrification of ammonium) across space and time. The role of microbially produced nitrate has become increasingly important over time in historical surface waters from the mid-90s to present – a trend that is coincident with increasing ammonium deposition to these systems.

1. Introduction

With a short growing season and poorly developed soils, alpine ecosystems are particularly vulnerable to environmental change. The role and impact of atmospheric nitrogen (N) deposition, one driver of environmental change, has been a focus of work at long-term research sites in the Colorado Rockies for decades (Baron and Campbell, 1997; Williams and Tonnessen, 2000; Burns, 2004). The resulting elevated N in Rocky Mountain alpine ecosystems has acidified soils and waters, altered C:N:P stoichiometry, and changed biodiversity and

primary productivity (Elser et al., 2009; Preston et al., 2016; Boot et al., 2016; Spaulding et al., 2015; Wolfe et al., 2001, 2003; Oleksy et al., in review). Landscape characteristics such as poorly developed soils, minimal vegetative cover, talus slopes, and bedrock allow for rapid infiltration of precipitation and low retention of N such that high nitrate concentrations are found in surface waters (Burns, 2004 and references within).

A significant portion of reactive N enters the environment through the emission of nitrogen oxides ($\text{NO}_x = \text{NO} + \text{NO}_2$) and ammonia (NH_3), which can be atmospherically transformed, transported and deposited as nitrate (NO_3^-) and ammonium (NH_4^+), respectively (e.g., Fowler et al., 2013). National trends reflect declines in NO_3^- deposition, while NH_4^+ concentrations are increasing in several regions of the country (e.g. the Midwest) (NADP, 2019; Li et al., 2016; Lloret and Valiela, 2016). While NO_3^- deposition in Rocky Mountain National Park (NADP site: CO98) has declined since 1995 (Schichtel et al., 2019) and in Niwot Ridge (NADP site: CO90), surface water concentrations remain elevated in alpine watersheds; stream NO_3^- export has actually increased in both Loch Vale (28%, 2000-2006) and Green Lakes Valley (40%, 2000-2009) (Baron et al., 2009; Mast et al., 2014; Barnes et al., 2014). A simultaneous increase in NH_4^+ deposition at these sites is also evident over the same time period compensating for the decline in NO_3^- and resulting in a negligible change in total inorganic N deposition (e.g., Schichtel et al., 2019). In fact, wet deposition of NH_4^+ and dry deposition of NH_3 dominate the total reactive N budget for wet and dry inputs (Benedict et al., 2013; Benedict et al., 2018), indicating atmospheric N inputs are still a problem.

Changes in the quality/form of atmospheric N deposition inputs in addition to climatic influences on a changing cryosphere and hydrology, likely act as important drivers of observed increases in surface water nitrate concentrations and fluxes. Increases in glacial and permafrost

thaw result in additional groundwater storage and late season source water supply appear to amplify during drought conditions that are associated with warming air and ground temperatures (Hill et al., 2008). Rock glaciers and permafrost are estimated to comprise as little as 16% and as much as 89% of catchment areas, and water storage estimates exceed volumes stored in snow glaciers in two proximal alpine watersheds in the Colorado Front Range (Clow et al., 2003; Hill et al., 2008). Consequently, increases in cryospherically-derived glacial reserves and/or thawing ice-rich permafrost soils have been hypothesized to contribute to elevated N concentrations in alpine watersheds via direct release of atmospheric NO_3^- , nitrification of released atmospheric NH_4^+ or remineralization of organic matter in thawing soils followed by nitrification (Saros et al., 2010; Baron et al., 2009; Barnes et al., 2014). Indeed, lakes fed by seasonal precipitation and glacial melt receive more NO_3^- than purely snow-fed lakes in the northern and central Rockies (Saros et al., 2010). Similarly, NO_3^- and NH_4^+ in snow glacier and rock glacier meltwater are distinctly higher in the Colorado Front Range than in the Cascades and Sierra Nevada containing similar glacial features (Fegel et al., 2016; Williams et al., 2016), which may reflect historically higher atmospheric N deposition in the Rocky Mountains compared to other mountain ranges (Fegel et al., 2016). As seasonal regime shifts in source-water occurs, there is a greater contribution from glacial thaw and an increasing trend in rock glacier NO_3^- concentrations as summer transitions to fall (Williams et al., 2006, 2007; Hafich, 2014; Baron et al., 2009; Heath and Baron, 2014; Barnes et al., 2014); these trends in late season flow and NO_3^- amplify during drought conditions (Williams et al., 2006; Hill et al., 2008). Evidence of nitrification activity in CO alpine soils include characterization of high soil NH_4^+ and nitrification rates within moist glacial outwash, talus and meadow soil types (Osborne et al., 2016), as well as, measurable and high N_2O emissions during N-fertilization alpine meadow experiments (Neff et al., 1994). While

glacial features have been shown to contribute to surface water NO_3^- concentrations, the role of legacy atmospheric N, present day depositional inputs, and biological processes (e.g., microbial) have not been well quantified. Therefore, it is important to understand the current role of atmospheric N deposition given the decline in direct atmospheric NO_3^- deposition (and increase in NH_4^+), and potential changes in the cryosphere that likely stimulate biological processes in these environments.

Stable isotopes of N and O in NO_3^- ($\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, and $\Delta^{17}\text{O}_{\text{NO}_3}$) present an opportunity to refine constraints on the provenance of NO_3^- in these alpine environments because they can help identify atmospheric versus microbially-produced NO_3^- . Compared to other NO_3^- sources, atmospheric NO_3^- is highly enriched in ^{18}O , and contains excess ^{17}O quantified as $\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O} \neq 0$ (Michalski et al., 2003). Typical values for NO_3^- deposited in mid to high latitudes range from 22-34‰ for $\Delta^{17}\text{O}$ and 25-115‰ for $\delta^{18}\text{O}$, while biological NO_3^- has a $\Delta^{17}\text{O}$ of 0‰ (Michalski et al., 2012 and references therein). Taking advantage of this, we investigated how atmospheric N deposition has changed over time and whether the input of atmospheric N directly and/or via thawing was responsible for elevated NO_3^- in high alpine lake surface waters. Nutrient concentrations (i.e., NO_3^- and NH_4^+) and stable isotopes of NO_3^- ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$) were used to characterize the complete isotopic composition of wet deposition inputs (i.e., snowpack, rain), glacial ice, glacial meltwater, and surface water samples from two alpine watersheds in the Colorado Front Range.

2. Materials and Methods

2.1 Sampling Sites

Both the Loch Vale watershed (40.2955, -105.6461) and upper Green Lakes Valley (40.0537, -105.6269) are alpine watersheds located in the Colorado Front Range east of the continental divide and west of the Denver-Boulder-Fort Collins corridor. These high elevation watersheds (3110 – 4009 m and 3515 – 4082 m, respectively) drain areas approximately 660 ha and 225 ha in size, respectively. Both watersheds are in seasonally snow-fed east- to northeast-facing granitic valleys and each catchment receives ~60-80% of wet precipitation as snow (Baron et al., 1992; Caine, 1995a). Catchment lakes (Sky Pond, Andrews Tarn, GL5 and GL4) are surrounded by steep talus slopes and rock walls. Loch Vale has two alpine sub-basins, Andrews Creek and Icy Brook. Both have glacial features (Andrews Glacier and Taylor Rock Glacier) that contribute meltwater to Andrews Tarn and Sky Pond (Baron et al., 1992). The upper basin in Green Lakes Valley is primarily alpine tundra with the GL5 Rock Glacier and Arikaree Glacier sitting near or at the head of the valley and two lakes, GL5 at the headwaters, draining into GL4 (Williams et al., 2007; Caine, 1995a-b).

Loch Vale is a popular hiking destination for outdoor enthusiasts in Rocky Mountain National Park. The headwaters of the Green Lakes Valley are located in the upper catchment of Boulder Creek, which supplies Boulder County with drinking water. Both Loch Vale (CO98, 40.2878, -105.6628 at 3159m) and Green Lakes Valley (CO90, 40.0367, -105.5440 at 3015m) have National Atmospheric Deposition Program-National Trends Network (NADP/NTN – referred to as NADP onward) collection sites to measure precipitation wet chemistry and amount (NADP, 2019, <http://nadp.slh.wisc.edu/>). From here on, Loch Vale and Green Lakes Valley will be referred to as LVWS and GLV, respectively.

2.2 Field Sampling

Samples were collected in 2017 and 2018 at the beginning (May-June) and end (late August/early September) of the summer season to try to capture maximum snow accumulation and baseflow conditions. Discrete sampling of a variety of sample-types took place, including: atmospheric wet deposition (snowpack and rain), glacial ice, glacial meltwater, and surface water. Samples were collected longitudinally from glacial features at the highest elevations to downstream lakes in each watershed. Prior to field work, all sampling equipment (e.g., sample bottles, syringes, ice samplers, etc.) was pre-cleaned with ultrapure (18.2 Ω) Milli-Q water.

Atmospheric deposition samples consisted of snow and weekly wet deposition (i.e., rain). Snow was sampled from ~1 m deep snow-pits (n=11) in both watersheds at multiple locations each year. Approximately 12 evenly distributed down pit samples were collected per pit. Precipitation samples from the two NADP sites from April through August in 2017 and 2018 were obtained from the NADP facility at University of Illinois (2017) and University of Wisconsin (2018). NADP samples were stored frozen until shipment to Brown University for analysis.

Glacial ice was sampled within the ablation zone (e.g. zone of mass loss) of Andrews Glacier in LVWS in both years to sample older, legacy atmospheric deposition. Weather conditions prevented complementary collections from Arikaree Glacier during both campaigns. Samples were collected opportunistically near the top and base of the ablation zone. Ice was sectioned in-situ using a pre-cleaned Kovacs corer in 2017 (Kovacs Enterprise, Mark III, 7.25 cm diameter). In 2017, the ice was wet, mushy, and fell apart as it came out of the auger barrel making it difficult to section ice precisely according to depth sampled. We used a handsaw, wooden wedges and a rock to sample blocks of ice using an alternative wedging approach in 2018 with better success sampling whole chunks of ice. The ice was also wet in 2018, but the

blocks sampled were intact and more easily sub-sampled in the field. All ice samples were handled using powder free gloves and transported in Ziploc bags in ice-filled coolers to the EcoCore Laboratory at Colorado State University within 8 hours of collection. Snow and ice samples melted at room temperature and filtered on vacuum pump filter towers using pre-combusted glass fiber filters (GFF) before being collected in pre-cleaned amber HDPE bottles. A liter of deionized water was used to rinse between samples. Filter system blanks were collected by filtering 250 mL of deionized water through pre-combusted GFFs; powder-free gloves and Ziploc bags were also tested for potential NO_3^- contamination.

Surface water was sampled from lake inflows/outflows and streams connecting lake networks. Glacial outlet meltwater and lateral glacial streams were sampled from both snow-fed and rock glacial inlet streams/rivulets following surface water collection methods described below. All surface water samples collected in-situ were filtered using syringe filters (Micropore, $0.45\mu\text{m}$), and stored in precleaned amber HDPE bottles. Prior to collection, bottle and syringe were rinsed three times with sample water, a mL of filtered water was discarded to remove any filter NO_3^- , and each bottle was filled with filtered water. This was done in triplicate. All snow, ice and surface water samples were stored frozen and shipped to Brown University for concentration and isotopic analyses.

Archived samples from Andrews Tarn and Sky Pond were compared with the 2017-2018 dataset from LVWS. These samples included surface water collected at the inlets of Andrews Tarn and Sky Pond between the years 1992-1996 and 2012-2013. Frozen archived samples were shipped frozen to Brown University for isotopic analysis. Previous characterization ($\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$) of 2012 GLV samples (e.g., snow, snowmelt, talus, surface and soil waters, groundwater, Hafich, 2014) were used for comparison with samples collected in 2017-2018.

2.3 Nutrient Concentration Analysis

Concentrations of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) were measured using a colorimetric nutrient analyzer (Westco SmartChem 200) and standard protocols (U.S. EPA Methods: 353.2 (Revision 2.0) for NO_3^- ; Method 354.1 for NO_2^- ; and Method 350.1 for NH_4^+). Analytical precision and accuracy were calculated using quality control replicates analyzed with each sample set: $\pm 0.5 \mu\text{mol L}^{-1}$ for NO_3^- , $\pm 0.2 \mu\text{mol L}^{-1}$ for NO_2^- and $\pm 0.6 \mu\text{mol L}^{-1}$ for NH_4^+ , and detection limits were 0.3, 0.2 and $1 \mu\text{mol L}^{-1}$, respectively. NADP wet precipitation is collected on a weekly basis. NADP sample concentrations are presented as volume-weighted monthly averages where the measured concentration was multiplied by the sample volume and divided by the total volume collected for the month.

2.4 Stable Isotopic Analysis

The isotopic composition of N ($\delta^{15}\text{N}_{\text{NO}_3}$ vs. N_2 in air) and oxygen ($\delta^{18}\text{O}_{\text{NO}_3}$ vs. Vienna Standard Mean Ocean Water or VSMOW) in NO_3^- was characterized using the bacterial denitrifier method (Sigman et al., 2001; Casciotti et al., 2002) on sample aliquots containing 20 nmol of NO_3^- . Briefly, denitrifying bacteria (*Pseudomonas aureofaciens*) were used to quantitatively convert NO_3^- to nitrous oxide (N_2O) in each sample. The sample gas was then extracted, purified and cryo-focused (collected in a trap submerged in liquid N_2) before analysis of mass ratios were determined on the gas chromatograph isotope ratio mass spectrometer (GC-IRMS, Thermo-Scientific Delta V Plus) in continuous flow mode. Isotopic mass ratios ($R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{18}\text{O}/{}^{16}\text{O}$) are reported in δ notation: (1) $\delta (\text{‰}) = [(R_{\text{sample}})/(R_{\text{standard}}) - 1] \times 1000\text{‰}$. Internationally recognized NO_3^- reference standards were used for sample calibration (Böhlke et al., 2003 and references therein). All reference standards were prepared in $18.2 \text{ M}\Omega$ water according to the concentration range of the samples being analyzed. Reproducibility for $\delta^{15}\text{N}_{\text{NO}_3}$

and $\delta^{18}\text{O}_{\text{NO}_3}$ was determined by calculating the pooled standard deviation of sample replicates (based on $n=36$ pairs; $\pm 0.3\text{‰}$ and $\pm 0.7\text{‰}$ for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$, respectively). Pooled standard deviations for $\delta^{15}\text{N}_{\text{NO}_3}$ standards IAEA-N3 ($n=114$) and USGS34 ($n=105$) were 0.1‰ and 0.2‰ , respectively. For $\delta^{18}\text{O}_{\text{NO}_3}$ standards, pooled standard deviations for IAEA-N3 ($n=110$), USGS34 ($n=107$), and USGS35 ($n=105$) were all 0.3‰ , respectively.

If enough sample remained following concentration and $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ analysis, 50 nmol of NO_3^- was converted to N_2O using denitrifying bacteria (*Pseudomonas aureofaciens*) and then decomposed to N_2 and O_2 using a heated gold tube at 770°C (Kaiser et al., 2007; Fibiger et al., 2013). Using the isotopic mass ratios of O_2 , $\Delta^{17}\text{O}_{\text{NO}_3}$ was derived using: $\Delta^{17}\text{O} = \delta^{17}\text{O} - 0.52 \times \delta^{18}\text{O}$ (Kaiser et al., 2007; Michalski et al., 2003). This relationship quantifies the mass-independent ^{17}O excess found in atmospheric trace gases that react with ozone (Kaiser et al., 2007; Thiemens and Heidenreich, 1983). Reproducibility for $\Delta^{17}\text{O}_{\text{NO}_3}$ is $\pm 0.4\text{‰}$ based on the pooled standard deviation of sample replicates ($n = 37$ pairs). Pooled standard deviation of $\Delta^{17}\text{O}_{\text{NO}_3}$ standards USGS34 ($n=101$) and USGS35 ($n=95$) were 0.8‰ and 1.0‰ , respectively.

One limitation of this analysis is the mass requirement and corresponding analytical volume limitation, which often results in few or no data points for low concentration/mass samples, such as those preserved in glacial ice. Our objective to characterize the isotopic composition of NO_3^- in glacial ice motivated the development of a new application of the denitrifier method using isotope dilution theory (Clark et al., in preparation). In brief, a NO_3^- reference standard was used to calibrate across a range of masses and a set amount of this same standard was added to a standardized volume of sample. If the mass-area relationship is well-constrained, the isotopic composition of the sample can be calculated using mass balance. Reproducibility was assessed using the standard deviation of the following in triplicate: 1) the

standardized spike additions for constraining the area-mass relationship (17.1 ± 1.2 volt-seconds or mass area units for 30 nmol additions), and 2) the isotopic composition of the reference standards and sample replicates (within 10-15% or ~ 2 -3‰). Using this technique, we improved our ability to analyze a subset of glacial ice samples ($n=15$) with very low concentrations (≤ 1 μM) that otherwise might not be measurable (Clark et al., in preparation).

An average $\Delta^{17}\text{O}_{\text{NO}_3}$ was calculated for snow and rain in each watershed by year ($\Delta^{17}\text{O}_{\text{average}}$). These average values were then used to discern the actual proportion of atmospherically oxidized NO_3^- present in all sample types (e.g., surface water, meltwater from rock and surface glaciers, ice, etc.), using the following equation from Michalski et al., (2004):

$$(2) \% \text{ Atmospheric Nitrate} = \Delta^{17}\text{O}_{\text{sample}} / \Delta^{17}\text{O}_{\text{average}} \times 100$$

Snow was used as the representative atmospheric sample type for this calculation in early summer (before mid-July) because it captures the isotopic composition of winter and spring precipitation accumulation that would be transported downstream during snowmelt. In contrast, a $\Delta^{17}\text{O}_{\text{NO}_3}$ average for rain was used to calculate % atmospherically derived nitrate (referred to as %AD from here on) in the late summer samples (after mid-July) to calculate estimates according to the atmospheric deposition sample type most prominent by season. This approach was conducted for each watershed and year to compensate for observed temporal and spatial differences in $\Delta^{17}\text{O}_{\text{NO}_3}$, rather than using one mean value for all calculations. Still, since a mean value was used to conduct this calculation (as opposed to a maximum), percentage calculations sometimes resulted in estimates slightly above 100% (evident primarily in snowpack samples, which had higher $\Delta^{17}\text{O}_{\text{NO}_3}$ compositions reflective of wintertime atmospheric conditions).

Unable to characterize $\Delta^{17}\text{O}_{\text{NO}_3}$ in precipitation samples from the same time period as the historical surface waters, the $\Delta^{17}\text{O}_{\text{NO}_3}$ of snow and wet deposition in LVWS during this study

(2017-2018) was assumed to be consistent with that in the 1990s and early 2000s. The $\Delta^{17}\text{O}_{\text{NO}_3}$ would only change significantly if important changes in the oxidizing budget of the atmosphere occurred, such that the major chemical formation pathways for NO_3^- in the atmosphere changed markedly. Even with recent emission changes, this does not appear to have occurred (Alexander et al., 2019). This assumption allowed us to use the mean $\Delta^{17}\text{O}_{\text{NO}_3}$ of snowpack and rain during these two years to quantify the % AD present in all historical surface water samples. Error was calculated using the standard deviation of the $\Delta^{17}\text{O}_{\text{NO}_3}$ and equation (2) for each sample type.

3. Results and Discussion

3.1 Inorganic nitrogen concentrations in Loch Vale Watershed and Green Lakes Valley

NO_3^- was present in all sample types collected each year from May to August in both watersheds (Figures 1, S1, S3). Sample types with the highest NO_3^- concentrations include: rock glacier meltwater and surface water followed by rain, glacial ice/meltwater and snowpack (Figure 1). NO_2^- was negligible in all samples. NH_4^+ concentrations were elevated in atmospheric samples, but very low (generally $<2\ \mu\text{M}$) or undetectable in surface waters (Figures 1, S1). Measurable NH_4^+ concentrations were highest in rain and lowest in snowpack (Figure 1). NADP rain samples had higher median NO_3^- and NH_4^+ concentrations ($\sim 11\ \mu\text{M}$ and $\sim 16\ \mu\text{M}$, respectively) relative to snowpack and glacial ice (Figures 1, S1). In contrast, snowpack had much lower median concentrations for NO_3^- and NH_4^+ ($\leq 3\ \mu\text{M}$ in LVWS and $\leq 6\ \mu\text{M}$ in GLV, respectively) (Tables S1-S2; Figure S1). Median concentrations for NO_3^- and NH_4^+ have declined in snowpack and wet deposition since the early 2000s, though overall ranges are largely consistent with previous observations (Clow et al., 2002; Nanus et al., 2018). Differences in concentration in snow/rain could reflect seasonal changes in transport of local or regional

sources of NO_3^- and NH_4^+ or how much of the atmospheric column is being washed out at these high elevations. Previous studies highlight the effect of elevation and upslope storms in determining ion concentration patterns (Wasiuta et al., 2015; Benedict et al., 2013), as well as post-depositional elution of snowpack solutes (Tsiouris et al., 1985; Naftz et al., 2011).

Glacial ice (LVWS) had very low NO_3^- concentrations compared to glacial meltwater sampled in GLV (median $<2.8 \mu\text{M}$ vs. $27.2 \mu\text{M}$ for both years, respectively) (Figure 1). While a broader range was observed in NH_4^+ (maximum of $\sim 15\text{-}18 \mu\text{M}$ for both years in each watershed), a low median (~ 5 and $6.3 \mu\text{M}$) was observed in both watersheds for glacial samples. Glacial meltwater (GLV) had considerably higher NO_3^- (median $=27.2 \mu\text{M}$) and comparable NH_4^+ values (median $=6.3 \mu\text{M}$) compared to the glacial ice sampled in LVWS (Figure 1a-d). NO_3^- concentrations were highest in rock glacier meltwater in both watersheds (Figures 1c-d, S1; median = LVWS ($21.1 \mu\text{M}$) and GLV ($38.6 \mu\text{M}$)), while NH_4^+ was $< 1 \mu\text{M}$ /negligible (Figure S1; Tables S1-S2). Rock glacier NO_3^- concentrations increased $\sim 2\text{-}3\times$ late into the summer season in GLV compared to early summer (e.g., 2017 and 2018 ranges: $33.7\text{-}64 \mu\text{M}$ and $26.7\text{-}77.5 \mu\text{M}$, respectively; Table S2). This is consistent with previous rock glacier meltwater observations in the GLV watershed (Williams et al. 2007; Hafich, 2014), as well as rock glacier meltwater characteristics across the western U.S. (including LVWS) (Fegel et al., 2016).

Surface waters exhibited high NO_3^- concentrations ($\sim 18 \mu\text{M}$ (LVWS); $9\text{-}14 \mu\text{M}$ (GLV)) throughout the summer season, while NH_4^+ was low/undetectable ($<1 \mu\text{M}$) in both watersheds (Figures 1a-d, S1; Tables S1-S2), consistent with previous studies (Campbell et al., 2002; Baron et al., 2009; Barnes et al., 2014). LVWS archival surface water samples reflect gradual decreases in mean NO_3^- concentration over time from 1992-1996 (mean $\pm$ SD: $24.2 \pm 8.9 \mu\text{M}$), 2012-2013

($21.5 \pm 11.9 \mu\text{M}$), and 2017-2018 (this study, $17.6 \pm 5.7 \mu\text{M}$ to $18.9 \pm 12.0 \mu\text{M}$) (Figure S2; Table S1).

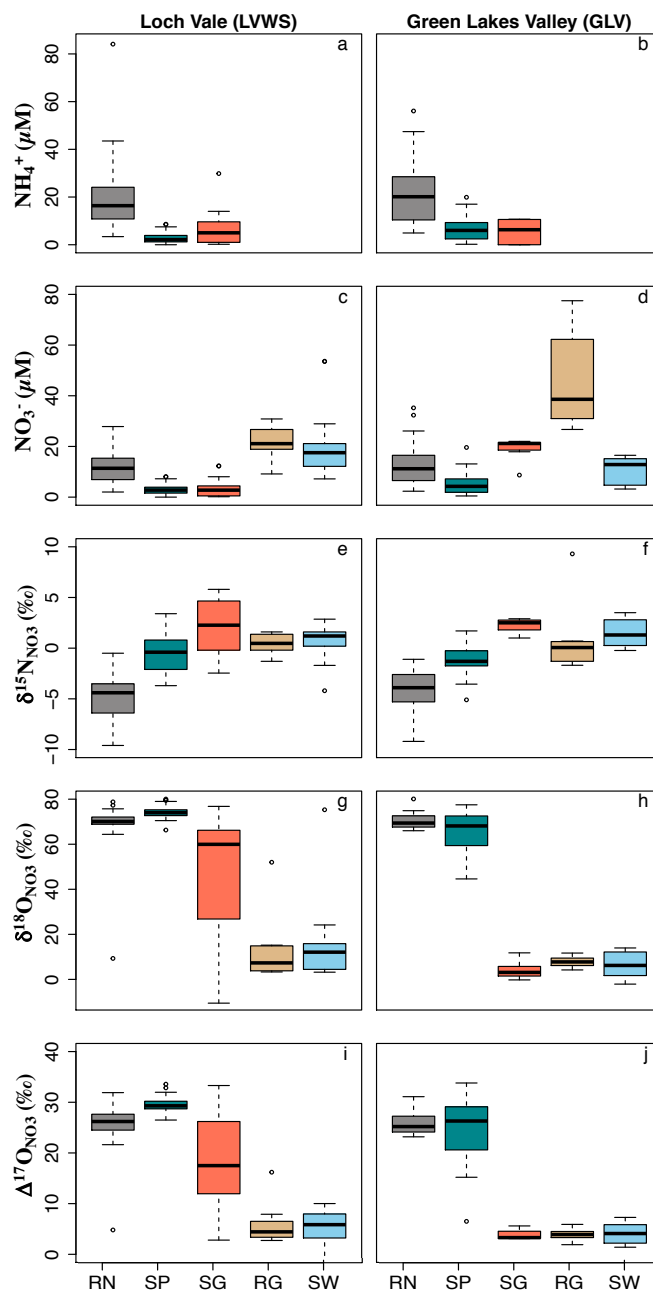


Figure 1: Median and range of all sample types, where (a-b) NH_4^+ concentration in $\mu\text{moles L}^{-1}$ (μM), (c-d) NO_3^- concentration in $\mu\text{moles L}^{-1}$ (μM), (e-f) $\delta^{15}\text{N}_{\text{NO}_3}$ (‰), (g-h) $\delta^{18}\text{O}_{\text{NO}_3}$ (‰) and (i-j) $\Delta^{17}\text{O}_{\text{NO}_3}$ (‰) reflected by sample type: rain (RN, LVWS (n=30); GLV (n=36)), snowpack (SP, LVWS (n=66); GLV (n=64)), surface glacial ice/meltwater (SG, LVWS (n=38); GLV (n=8)), rock glacier meltwater (RG, LVWS (n=27); GLV (n=13)), and surface water (SW, LVWS (n=51); GLV (n=15)) for LVWS and GLV for early and late summer in 2017-2018.

3.2 Isotopic composition of NO_3^- results

NO_3^- in snowpack and rain typically reflected low $\delta^{15}\text{N}_{\text{NO}_3}$ (-8.0 to 3.4‰) and very elevated $\delta^{18}\text{O}_{\text{NO}_3}$ (44.6 to 80.1‰) for both watersheds (Figure 1e-h, Tables S1-S2). Rain (representative of late spring and summer wet deposition) had more negative $\delta^{15}\text{N}_{\text{NO}_3}$ values (-8.0 to -1.7‰) than winter snowpack (-5.1 to 3.4‰) and a smaller range in $\delta^{18}\text{O}_{\text{NO}_3}$ (65.2 to 76.6‰) compared to snowpack (44.6 to 80.1‰) (Figure 1e-h, Tables S1-S2). Snow samples were more enriched with respect to ^{15}N compared to rain, potentially reflecting seasonal differences in precursor NO_x sources (Clow et al., 2015). The range of wet deposition $\delta^{15}\text{N}_{\text{NO}_3}$ values was larger and more depleted with respect to ^{15}N for samples collected in this study compared to previous work in the region (see Table S3), but $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ compositions for these atmospheric sample types are consistent with other mid-latitude mountain studies (Table S3). Distinctions in $\delta^{18}\text{O}_{\text{NO}_3}$ between winter snowpack and summer precipitation reflect seasonal differences in atmospheric oxidation chemistry prior to deposition (Michalski et al., 2012, 2003; Hastings et al., 2003). $\Delta^{17}\text{O}_{\text{NO}_3}$ values for NADP rain and snowpack ranged from 21.8 to 31.3‰ and 6.5 to 36.2‰, respectively, where snowpack $\Delta^{17}\text{O}_{\text{NO}_3}$ (and $\delta^{18}\text{O}_{\text{NO}_3}$ see above) composition spanned a much larger range, especially in GLV (Figure 1i-j, Table S1-S2). Previous $\Delta^{17}\text{O}_{\text{NO}_3}$ characterization of GLV snow samples were consistent with high values expected for atmospheric sample types (i.e., 22-34‰) and previous work in the region, but did not capture the entire $\Delta^{17}\text{O}_{\text{NO}_3}$ range in the current study (specifically 2018 when values between 6.5-22‰ were observed, Table S3).

Given the conditions of the ice (i.e., soft, mushy), difficulty of sampling (e.g., close to high noon instead of early morning) and limited sample size, the objective was to characterize a bulk ice geochemical signature. Glacial ice values covered a broad range across all isotopes;

$\delta^{15}\text{N}_{\text{NO}_3}$ was generally positive (-2.5 to 5.8‰), while $\delta^{18}\text{O}_{\text{NO}_3}$ (59.6 to 72.1‰) and $\Delta^{17}\text{O}_{\text{NO}_3}$ (2.8 to 33.2‰) were generally very high and consistent with typical snow values, however, there were some lower $\Delta^{17}\text{O}_{\text{NO}_3}$ values than expected if ice were merely stored atmospheric deposition (Figure 1e,g,i; Table S1). $\delta^{15}\text{N}_{\text{NO}_3}$ in glacial ice was slightly more enriched compared to meltwater, snow and rain in both watersheds (Figure 1e-f, Tables S1-S2). Glacial meltwater samples exhibited a narrower $\delta^{15}\text{N}_{\text{NO}_3}$ range compared to glacial ice (Figure 1e-f; Tables S1-S2). $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ in glacial meltwater spanned a larger range in LVWS ($\delta^{18}\text{O}_{\text{NO}_3}$ = 7.0 to 38.4‰; $\Delta^{17}\text{O}_{\text{NO}_3}$ = 4.3 to 17.9‰) compared to GLV ($\delta^{18}\text{O}_{\text{NO}_3}$ = -0.2 to 5.8‰; $\Delta^{17}\text{O}_{\text{NO}_3}$ = 2.7 to 4.6‰) (Figure 1g-j, S2; Tables S1-S2). Specifically, there were more glacial samples that had low $\Delta^{17}\text{O}_{\text{NO}_3}$ in LVWS than in GLV glacial meltwater. In 2018, a lateral meltwater stream between Andrews Glacier and underlying bedrock reflected $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ isotopic values between all other atmospheric and surface water samples (i.e., $\delta^{18}\text{O}_{\text{NO}_3}$ = 15 to 40‰; $\Delta^{17}\text{O}_{\text{NO}_3}$ = 8 to 18‰) (Figure S3b-c). No previous glacial ice (or meltwater in LVWS) samples have been characterized isotopically in this region for comparison, but a Wind River Range (Wyoming) glacier study observed a more negative range in $\delta^{15}\text{N}_{\text{NO}_3}$ (-5.9 to -3.2‰) from 1950 to present day, which was suggested to exhibit an anthropogenic signature (Naftz et al., 2011). Interestingly, this observed range does not overlap with the $\delta^{15}\text{N}_{\text{NO}_3}$ observed in the current study (i.e., -2.5 to 5.8‰). In addition, a study characterizing subglacial and supraglacial runoff/meltwater for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ in Svalbard found supraglacial samples (at the surface) were more representative of atmospheric NO_3^- and subglacial samples (near the glacier bed) reflected a biological isotopic signature (Table S4). While consistencies are apparent between studies, we observed a much broader range in N and O isotope mass ratios (Table S4), indicating that the glacial samples collected reflect a mix of atmospheric and terrestrially sourced NO_3^- .

Considered an abiotic environment until recently, it is now clear that glaciers have active N cycles (Tranter et al., 2004; Edwards et al. 2011; Telling et al., 2011; Hodson et al., 2005; Wynn et al., 2007) and contain diverse microbial communities and microhabitats influenced by supra-/sub-glacial processes (Anesio et al., 2017; Anesio and Laybourn-Parry, 2012; Ren et al., 2019; Havig and Hamilton, 2019).

Surface water samples all exhibited low $\Delta^{17}\text{O}_{\text{NO}_3}$ compositions, while $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ compositions were lower and higher compared to the atmospheric sample types measured, respectively (Figure 1e-j). In LVWS and GLV, surface water and rock glacier samples generally shared overlapping isotope characteristics across sample years (LVWS: $\delta^{15}\text{N}_{\text{NO}_3} \sim -1.7\text{‰}$ to 2.9‰ , $\delta^{18}\text{O}_{\text{NO}_3} = 0.9$ to 24.2‰ , $\Delta^{17}\text{O}_{\text{NO}_3} = -0.7$ to 10.0‰ ; GLV $\delta^{15}\text{N}_{\text{NO}_3} = -1.7$ to 3.5‰ with a possible outlier of 9.3‰ , $\delta^{18}\text{O}_{\text{NO}_3} = -2.1$ to 14.0‰ , $\Delta^{17}\text{O}_{\text{NO}_3} = 1.4$ to 7.3‰) (Figures 1c-f, S3; Tables S1-S2). These overlapping ranges between sample types are largely consistent with previous findings from each watershed (Table S3), however observed ranges for $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ were larger for rock glacier and surface water sample types in LVWS than in GLV (Tables S1-S2). Rock glacier NO_3^- concentrations in GLV increased from early to late summer while $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ remained consistent at $\sim 8\text{‰}$ and 4‰ the whole season, respectively (Table 1, Figure S3e-f).

LVWS archival surface water samples collected in 1992-1996 and 2012-2013 exhibited comparable ranges and mean values for all isotopes (Figure S2). However, on average, the $\delta^{15}\text{N}_{\text{NO}_3}$ in historical samples (mid-90s: $1.9 \pm 1.2\text{‰}$, early 2010s: $1.4 \pm 1.7\text{‰}$) was more elevated and $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ exhibited larger ranges compared to the present study indicating a greater presence of uncycled atmospheric NO_3^- in the past and a shift in sources (e.g. greater contribution from the cryosphere or atmospheric NH_4^+) through time (Tables S1-S2, Figure S2).

All LVWS surface water samples (1992-1996, 2012-2013, and 2017-2018) isotopically characterized in this study compare well with previously observed ranges for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ in LVWS (Campbell et al., 2002), but the characterization of $\Delta^{17}\text{O}_{\text{NO}_3}$ enables for a more quantitative estimate of atmospheric NO_3^- in these surface waters (via %AD) than $\delta^{18}\text{O}_{\text{NO}_3}$ alone.

Much attention has been paid to the isotopic composition of NO_3^- in lake surface waters in national parks across the Rocky Mountains (e.g., Glacier, Grand Teton, Great Sand Dunes, Rocky Mountain, and Yellowstone) and the Uinta Mountains (Nanus et al., 2008 and references therein; Hundey et al., 2016) in an attempt to link $\delta^{15}\text{N}_{\text{NO}_3}$ to emission sources, although overlapping source ranges can make apportionment a challenge. Recent work has improved constraints on direct NO_x source emissions, including vehicles, soils and biomass burning, which will improve the ability to attribute source values to the isotopic composition of receiving waters in the future (Miller et al., 2017; Miller et al., 2018; Fibiger and Hastings, 2016; Chai et al., 2019). Below, we focus on the significance and utility of $\Delta^{17}\text{O}_{\text{NO}_3}$ to quantify and assess atmospheric inputs across the alpine landscape.

3.3 Persistent atmospheric NO_3^- supply in space and time

Climate is an important lever on a mountain watershed's biogeochemical mass balance (Heath and Baron, 2014). Warming and drought influence the sources, storage, and lags between inputs from deposition and loss from the catchment. Enhanced N export in streams could reflect important changes in the cryosphere and hydrologic connectivity under warming summer temperatures rather than atmospheric N deposition (Baron et al., 2009; Barnes et al., 2014). As glacial relics continue to shrink, the impact of discharging large pulses of legacy pollution/nutrients on downstream processes remain uncertain (Milner et al., 2017).

All samples characterized here indicate a presence of atmospheric NO_3^- , as evidenced by a nonzero $\Delta^{17}\text{O}_{\text{NO}_3}$ composition. As expected, high $\Delta^{17}\text{O}_{\text{NO}_3}$ values are associated with all atmospheric sample types (e.g., snowpack and rain), while all other sample types (e.g., surface water) exhibit lower $\Delta^{17}\text{O}_{\text{NO}_3}$ values especially in late summer. Perennial $\Delta^{17}\text{O}_{\text{NO}_3}$ composition suggests that atmospheric NO_3^- is present and supplied throughout the summer season, well past snowmelt. During this seasonal progression, the overall NO_3^- concentration contributed by rock glacier inputs increases 2-3x, while $\Delta^{17}\text{O}_{\text{NO}_3}$ values are sustained ($\sim 4\text{‰}$); this is especially apparent in the rock glacier meltwater in GLV (Figure 2; Table S5). These low $\Delta^{17}\text{O}_{\text{NO}_3}$ values implicate the role of NO_3^- produced by mass-dependent processes (i.e., biological/microbial transformations). The production of NO_3^- via microbial nitrification incorporates oxygen atoms from both atmospheric oxygen and water (Mayer et al., 2001), which both have $\Delta^{17}\text{O}=0\text{‰}$ (Michalski et al., 2004), and thus dampens the high $\Delta^{17}\text{O}_{\text{NO}_3}$ signature of atmospheric NO_3^- . In contrast, denitrification is a process that consumes NO_3^- (i.e., lowering the concentration) resulting in enrichment of ^{15}N and ^{18}O , leaving the $\Delta^{17}\text{O}_{\text{NO}_3}$ unchanged.

The relationship between $\Delta^{17}\text{O}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ lends additional evidence that nitrification contributes a major supply of microbially-produced NO_3^- to these systems. Based on the relationship between $\delta^{18}\text{O}_{\text{NO}_3}$ and $\Delta^{17}\text{O}_{\text{NO}_3}$ across all sample types, we can estimate the $\delta^{18}\text{O}_{\text{NO}_3}$ of the biological source (i.e., the y-intercept where $\Delta^{17}\text{O}_{\text{NO}_3} = 0\text{‰}$) (Michalski et al., 2004; Bourgeois et al., 2018; Hafich 2014; Darrouzet-Nardi et al., 2012). Using this approach, we find negative $\delta^{18}\text{O}_{\text{NO}_3}$ values (-5.1‰ LVWS; -3.6‰ GLV) for nitrification, in line with the expected range ($\delta^{18}\text{O}_{\text{NO}_3} = -5$ to 15‰ ; Mayer et al., 2001), as well as previous estimates in the study region (-4.7‰ , Hafich, 2014). The $\delta^{18}\text{O}_{\text{NO}_3}$ values also share some overlap with soil water and groundwater samples previously characterized in GLV (Table S3).

These observations suggest a large supply of non-atmospheric NO_3^- must comprise ~80% of the NO_3^- exported from these high elevation sites (Table S5). Further, the proportional contribution does not change dramatically seasonally, especially in GLV where rock glacier inputs maintain a consistent signature (i.e., $\Delta^{17}\text{O}_{\text{NO}_3} \sim 4\text{‰}$) despite the significant increase in concentration late in the season (Figures 2, S3f). While this corroborates previous evidence of microbial nutrient sources in each respective watershed (Williams et al., 2007; Hafich, 2014; Campbell et al., 2002), the fact that uncycled atmospheric NO_3^- is continually present, even late in the season, means we need to rethink nitrogen sources and processing within these alpine catchments.

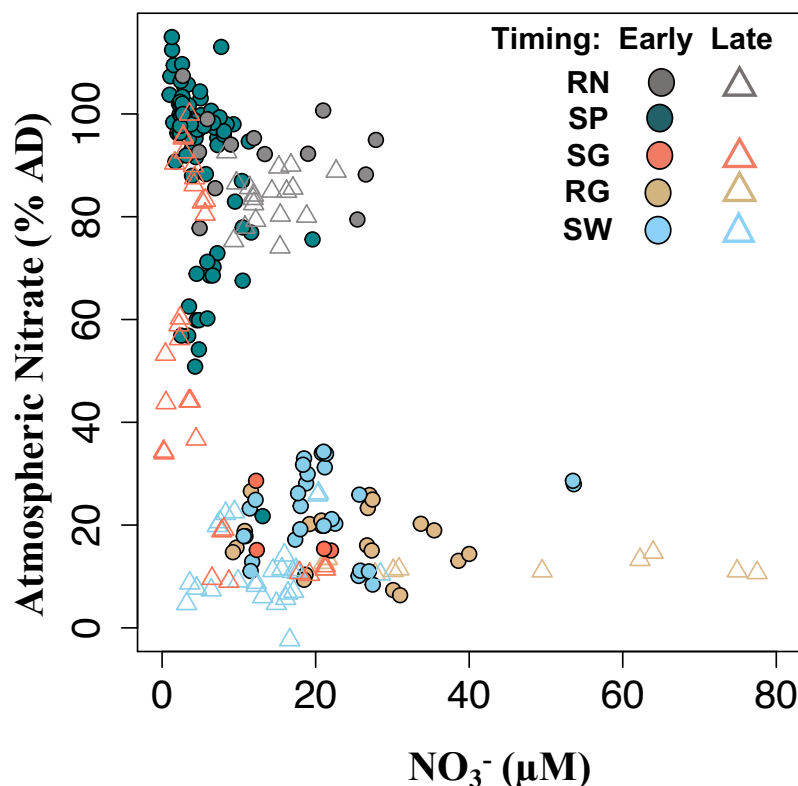


Figure 2: NO_3^- concentration in $\mu\text{moles L}^{-1}$ (μM) vs. percent atmospheric NO_3^- deposition (%AD) reflected by sample type: surface glacial ice/meltwater (SG), rain (RN), rock glacier meltwater (RG), snowpack (SP), and surface water (SW) for LVWS and GLV for 2017-2018 where circles and triangles represent early and late summer sample collections, respectively.

Interestingly, atmospheric NO_3^- is entering surface waters beyond the ionic pulse of nutrients associated with snowmelt. This is surprising considering nutrient delivery in alpine systems is thought to be heavily reliant on seasonal hydrology (Sickman et al., 2003; Williams and Melack, 1991) and winter snowpack NO_3^- concentrations are much lower ($<10\mu\text{M}$) than those observed in receiving surface waters (on the order of $20\mu\text{M}$). Based on these findings and the consistent supply of atmospheric NO_3^- late in the summer (Figures 2-3), glacial features and/or rain must contribute to surface water composition. These findings support previous work that used solute chemistry to draw similar conclusions (Williams et al., 2007; Saros et al., 2010), but the isotopic compositions presented in this study offer additional insights. The N and O isotopic composition of NO_3^- in rain and snowpack (with the exception of snowpack $\delta^{15}\text{N}_{\text{NO}_3}$) share no overlap with surface water samples collected (Figure 1c-f). In contrast, surface water NO_3^- compositions share similar isotopic signatures as glacial features (Figure 1c-f). This strongly suggests that glacial meltwater, containing ‘uncycled’ atmospheric NO_3^- , is a critical component to the observed elevated NO_3^- concentrations, supplying an important source of nutrients to downstream aquatic systems at a minimum late in the summer season but likely throughout the growing season.

3.4 Quantifying seasonal and long-term trends in atmospheric NO_3^- contribution

We can address whether the contribution of atmospheric NO_3^- has in fact changed over time or whether a non-atmospheric/microbial source of NO_3^- plays a larger role in the present budget by quantifying the proportional contribution of atmospheric NO_3^- to the surface water composition in these alpine ecosystems across space and time using $\Delta^{17}\text{O}_{\text{NO}_3}$ as a conservative tracer. Using seasonal constraints on $\Delta^{17}\text{O}_{\text{NO}_3}$, we estimate the proportional contribution of atmospheric and non-atmospheric (e.g., microbial) NO_3^- for all samples collected in 2017-2018

and LVWS historical surface water samples. For historical samples, mean values for snowpack (~30‰) and rain (~25‰) were used for this calculation and the calculated error was $\pm 3-9\%$. Snowpack and rain have expectedly high %AD (typically 80-100%) compared to surface water (~5-35%) and glacial sample types (rock glacier ~5-25% vs surface glacier ~10-100%) (Figure 2). Atmospheric $\Delta^{17}\text{O}_{\text{NO}_3}$ characterized in archived (mid 1990s and early 2010s) and more recent (2017-18) LVWS surface water samples share overlapping ranges (Figure S2), which may suggest an unchanged supply of atmospheric NO_3^- in these systems. In contrast, on a shorter time scale there is a decline in %AD in surface water relative to atmospheric and glacial inputs seasonally (Figure 2).

As water sources transition from snowmelt (early summer) to baseflow (late summer and early fall), the contribution of atmospheric NO_3^- to surface waters declines. Proportionally, atmospheric NO_3^- (%AD) only represents as much as 20% (32%) in early summer and as little as 11% (25%) in late summer for rock glacier-fed (and surface glacier-fed systems), respectively (Figure 3), and appears consistent between both watersheds (Figure 2-3; Table S5). The vast majority (75-89%) of NO_3^- exported is non-atmospheric (i.e., microbially-transformed) by the end of summer in surface and rock glacier-fed systems (Figure 2), indicating that atmospheric NO_3^- is not a driver of these elevated concentrations and that the microbially-transformed NO_3^- source dominates, regardless of type (rock-glacier fed vs glacial-fed) and extent of cryospheric features, late in the thaw season to baseflow conditions (Figure 3).

Historical surface waters reflect the same seasonal shift in atmospheric NO_3^- present as summer progresses (Figure 3), which align with seasonal changes in hydrology. Interestingly, these samples also reflect declines in atmospheric NO_3^- spanning ~3 decades (Figure 3) and correspond with declining NO_3^- deposition trends observed at national and local scales over the

same time period (NADP, 2019; Schichtel et al., 2019). While the proportional contribution of atmospheric NO_3^- was higher in the past, microbially-cycled NO_3^- remains the dominant source dating back to the mid-90s (Figure 3). The prominence of this cycled NO_3^- source is consistent with previous estimates ($\geq 75\%$) in LVWS using $\delta^{18}\text{O}_{\text{NO}_3}$ in the mid-90s and $\sim 81\%$ in GLV in 2012 using $\Delta^{17}\text{O}_{\text{NO}_3}$ (Campbell et al., 2002 and Hafich, 2014, respectively).

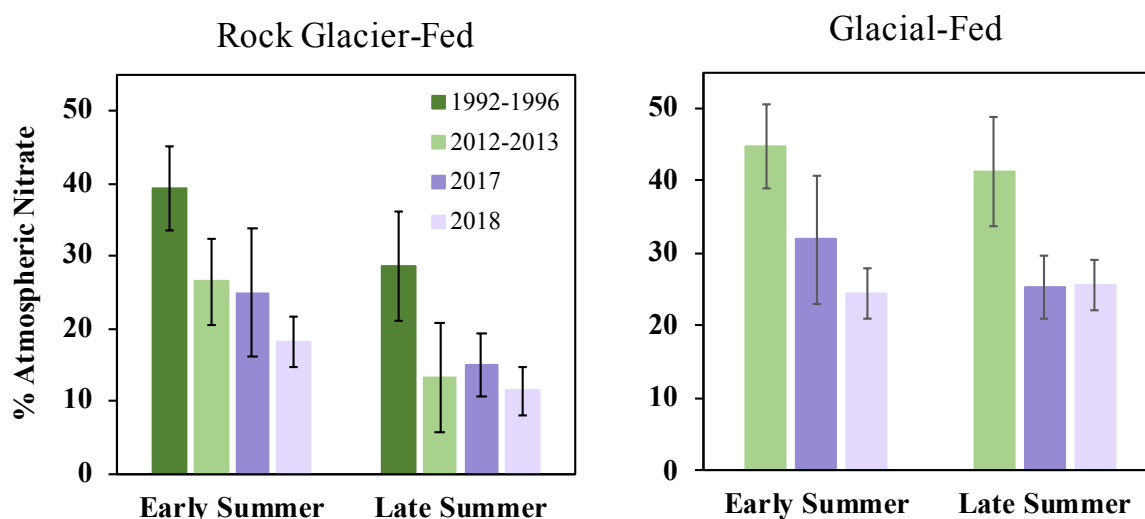


Figure 3: Percent atmospheric NO_3^- in LVWS surface waters from the mid-90s, early 2010s, and this study in early and late summer. This calculation was conducted for lake surface waters fed by rock glacier (Sky Pond) and ice glacier (Andrews Tarn) meltwater using mean values for 2017-2018 snowpack ($\sim 30\%$) and rain ($\sim 25\%$). Error bars represent the standard deviation of the $\Delta^{17}\text{O}_{\text{NO}_3}$ average presented as percent atmospheric NO_3^- ($\pm 3-9\%$, based on seasonally dominant precipitation sample types).

3.5 Implications of this work

A source of microbially-cycled (i.e., nitrified) NO_3^- has a dominant presence in these alpine systems over the span of three decades. These findings have implications for our understanding of how these environments evolve under a changing climate and the amenable conditions for biological processes to occur. For example, increasing air temperatures leading to glacial retreat (e.g. Caine, 2010), may shift the systems from snow to rain-dominant precipitation

regimes (Knowles et al., 2006) and enhanced dry deposition can enhance thaw rates due to albedo changes (Skiles et al., 2012), create slushy ice conditions, and providing more moisture for microbial activity to take place. Glacial reservoirs (as well as high elevation snowfields) contain nutrient sources for microbial processes such as legacy and modern atmospheric N (wet and dry deposition), organic matter, black carbon, dust, or clay-bound NH_4^+ (Fegel et al., 2019; Hood et al., 2015; Li et al., 2019; Wynn et al., 2007), and are more biologically active than previously thought (Ren et al., 2019; Hamilton and Havig, 2018; Boetius et al., 2015). Further snow acts as an insulator over microbially active soils during winter, which utilize NH_4^+ from precipitation to nitrify NO_3^- prior to being flushed downstream during hydrologically active parts of the year (i.e., peak snowmelt) (Williams et al., 1996; Brooks et al., 1996). In addition, snowpack can act as a biogeochemical and photochemical reactor that actively oxidizes clay-bound NH_4^+ to form NO_3^- in addition to emitting NO_x (Amoroso et al., 2010). Given the broad range we observe in $\Delta^{17}\text{O}_{\text{NO}_3}$, nitrification within Colorado snowpack could become more important to the cycling of inorganic N deposition downstream, as well as, the release of gaseous species to the atmosphere in the future, but additional study is needed.

As precipitation regimes and glaciers continue to change, the proportion of NO_3^- from glacial inputs entering alpine watersheds is almost certainly going to remain prominent until the distribution of cryosphere features is eliminated entirely. Based on forecasted warming scenarios, air and ground temperature measurements, thawing of surface (snow glaciers) and subsurface ice (i.e., permafrost, rock glaciers) is expected to accelerate in alpine systems in the Colorado Front Range (Christiansen et al., 2007; Hill et al., 2008) – a process that enhances late season water flow in glaciated catchments, compensating for minimal water supply during low snow years. It remains to be seen whether direct melt inputs of unaltered atmospheric deposition

or hydrologic flushing of microbially active habitat (e.g., soils/subglacial) will be the dominant driver in the future, but currently atmospheric NO_3^- (as direct deposition and glacial stores) is not the dominant supply of NO_3^- to these surface waters. Continuous changes in the cryosphere are likely to result in fluctuations in the geochemical inputs to downstream ecosystems, but the dominance of biological/microbial processes (i.e., nitrification) that produce NO_3^- in these watersheds has been consistent over time.

Nitrification requires a supply of NH_4^+ . If atmospheric NH_4^+ deposition continues to increase (Schichtel et al., 2019; NADP, 2019), it is likely that biological activity in these environments will increase in response. Additionally, if atmospheric NH_4^+ is a key driver to the sustained export of NO_3^- , then atmospheric N deposition remains problematic despite NO_x regulations. Agricultural and urban sources of $\text{NH}_3/\text{NH}_4^+$ are becoming more prominent generally east of the Colorado Front Range (Li et al., 2016; Benedict et al., 2013), while natural NH_3 emissions from subalpine grassland and forest soils have been determined to be low in Rocky Mountain National Park (Stratton et al., 2018). Remineralization of dissolved organic matter (DOM) released by glacial meltwater or organic matter in newly thawed soils may supply an additional source in future climates. Recent work suggests that rock and surface glaciers supply DOM sources that vary in quality and quantity in high elevation alpine lakes (Fegel et al., 2019). While we are unable to assess the significance of soil or glacial organic matter directly, we can roughly estimate whether enough atmospheric NH_4^+ is entering these systems to supplement the microbially-produced NO_3^- proportion.

Almost 90% of the NO_3^- inventory entering these lakes is microbially-generated by late summer, which begs the question: where is it all coming from? Could it come from atmospheric deposition alone? Ammonium concentrations are elevated in atmospheric inputs relative to

surface waters (where NH_4^+ is not detected) suggesting that any incoming supply of reduced N is preferentially taken up and/or oxidized. Under the assumption that the conversion of NH_4^+ to NO_3^- is 1:1 with no quantitative loss, we can roughly estimate the concentration of NH_4^+ required to produce enough microbially-produced NO_3^- to dilute the atmospheric NO_3^- supply using the %AD of the microbial source and the concentration of the input. For example, using a maximum observed NO_3^- concentration (e.g., $74\mu\text{M}$) and the calculated %AD (e.g., AD=11%, thus non-AD=89%), we can estimate the concentration of NO_3^- (produced by microbial activity) entering via rock glacier inputs (e.g., $\sim 66\mu\text{M}$). On average, 1.5x less NO_3^- enters these systems as wet precipitation relative to NH_4^+ ($\sim 30\text{--}40\mu\text{M}$ and as high as $55\mu\text{M}$ for NH_4^+ alone), and the amount of NO_3^- and NH_4^+ entering via snowpack is $\sim 1:1$ and on the order $\leq 10\mu\text{M}$, respectively (Figures 1c-d, S1). Based on this calculation and concentrations of seasonal wet deposition and snowpack inputs, nitrification activity could be entirely sourced from atmospheric NH_4^+ .

Additional constraints on the source of NH_4^+ supplying nitrification activity could aid in determining how significant of a role atmospheric versus non-atmospheric sources of NH_4^+ (e.g., OM from melting glaciers or thawing permafrost) are in these evolving alpine environments. However, it is likely that atmospheric NH_4^+ delivered via wet deposition plays an important role as it is supplied during periods of peak productivity during the summer period, practically negligible in concentration in all non-atmospheric sample types and has been increasing over time. NH_3 emissions are unregulated in the United States and will continue to contribute to the persistence of NH_4^+ deposition and loading of this highly desirable nutrient to surface waters. If the current reactive N depositional landscape remains unchanged, the health of aquatic and terrestrial ecosystems in the Colorado Front Range may be further affected.

Overall, results suggest:

- 1) Atmospheric NO_3^- deposition contributes only 20-11% of the surface water NO_3^- in two alpine systems of Colorado, down from a contribution as high as 40-45% in the 1990s;
- 2) Non-atmospheric supplies of NO_3^- have been the dominant source of NO_3^- to alpine surface waters across space (i.e., LVWS and GLV) and time (i.e., 1992-1996, 2012-2013, 2017-2018, early-late summer) collectively covering the 30-year window when major shifts in atmospheric reactive nitrogen emissions have taken place;
- 3) Increasing NH_4^+ in atmospheric deposition could be a source of this sustained NO_3^-

4. Acknowledgements

This work was funded by: the Natural Science Division at Colorado College, Brown Graduate School, Institute at Brown for Environment and Society, and National Science Foundation (Grant Number: OCE-1433989 to M.G.H.). Special thanks to all of the volunteers that assisted with fieldwork operations through the following organizations/institutions including: National Park Service, CU-Boulder, Colorado College, Colorado State University, Denver University and Brown University. Many thanks to the following people for their help with permitting, field/lab logistics and project support in Colorado and Rhode Island: Tim Weinmann, Daniel Bowker, Natural Resource Ecology Lab, Scott Esser, Jennifer Morse, Bill Bowman, the Mountain Research Station Niwot Staff, Jake Ritter & Trey, Emily Joyce, Ruby Ho, Joseph Orchard, Dave Murray, Nora Richter, Anson Cheung, Celia Hack, Claire Bekker, and the Hastings Lab. Thanks to Robert Hawley, Zoe Courville, and Eric Osterberg for generously providing their equipment, guidance and expertise on ice sampling. Lastly, we thank the National Atmospheric Deposition Program for providing publicly available data and site sample splits.

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6. Supplement to: Quantifying atmospheric nitrogen deposition in alpine ecosystems of the Colorado Front Range

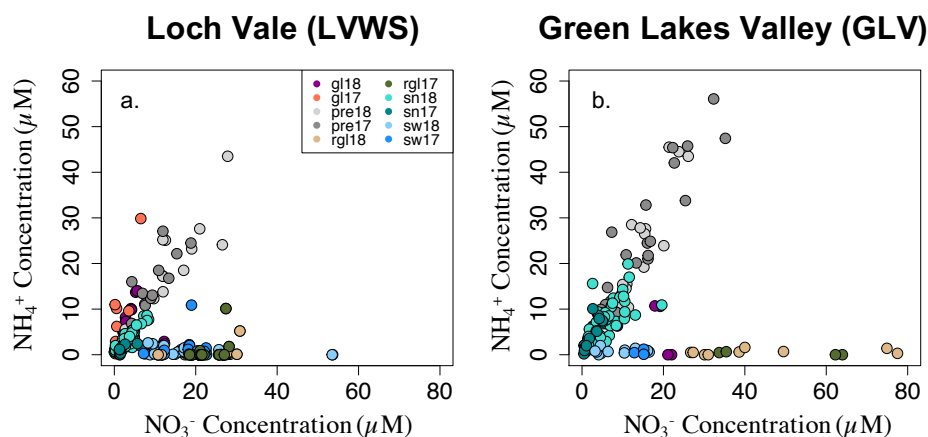


Figure S1: NO_3^- and NH_4^+ concentrations in $\mu\text{moles L}^{-1}$ (μM) reflected by location (e.g., a.) Loch Vale and b.) Green Lakes Valley), collection year (2017, 2018) and sample type: glacial (gl), rain (pre), rock glacier (rgl), snow (sn), and surface water (sw).

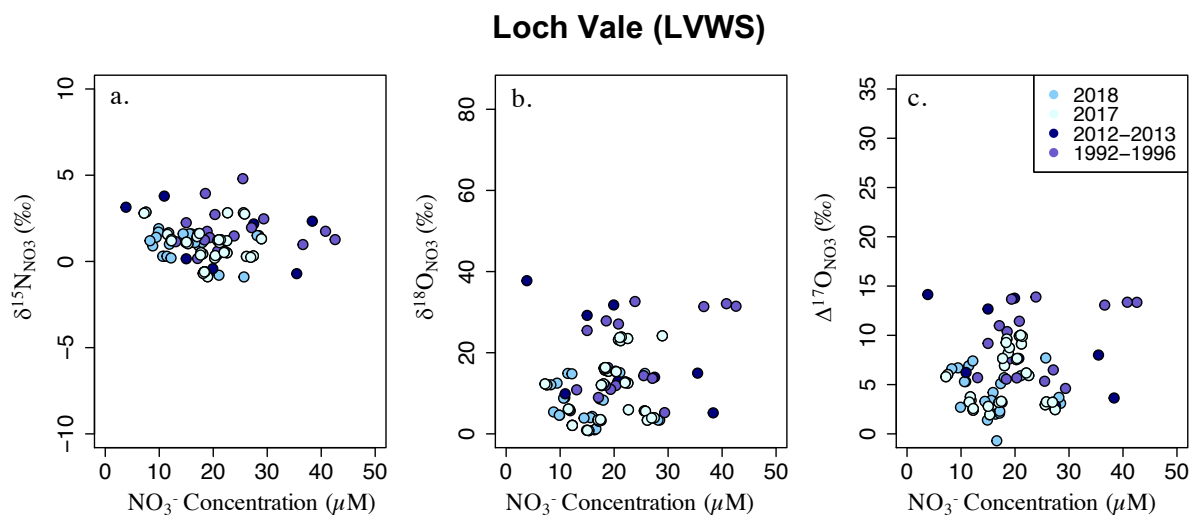


Figure S2: $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$ vs. NO_3^- concentration (μM or $\mu\text{mol L}^{-1}$) for Loch Vale Watershed surface water samples collected in 1992–1996, 2012–2013, 2017 and 2018. Samples collected before 2017 were sampled by the USGS Loch Vale Watershed Monitoring Program. The mean $\pm$ stdev for $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$ for the mid-90s and early 2010s, respectively: (1.9 $\pm$ 1.2‰; 19.5 $\pm$ 9.7‰; 9.4 $\pm$ 3.5‰) and (1.4 $\pm$ 1.7‰; 19.5 $\pm$ 11.8‰; 8.6 $\pm$ 4.4‰).

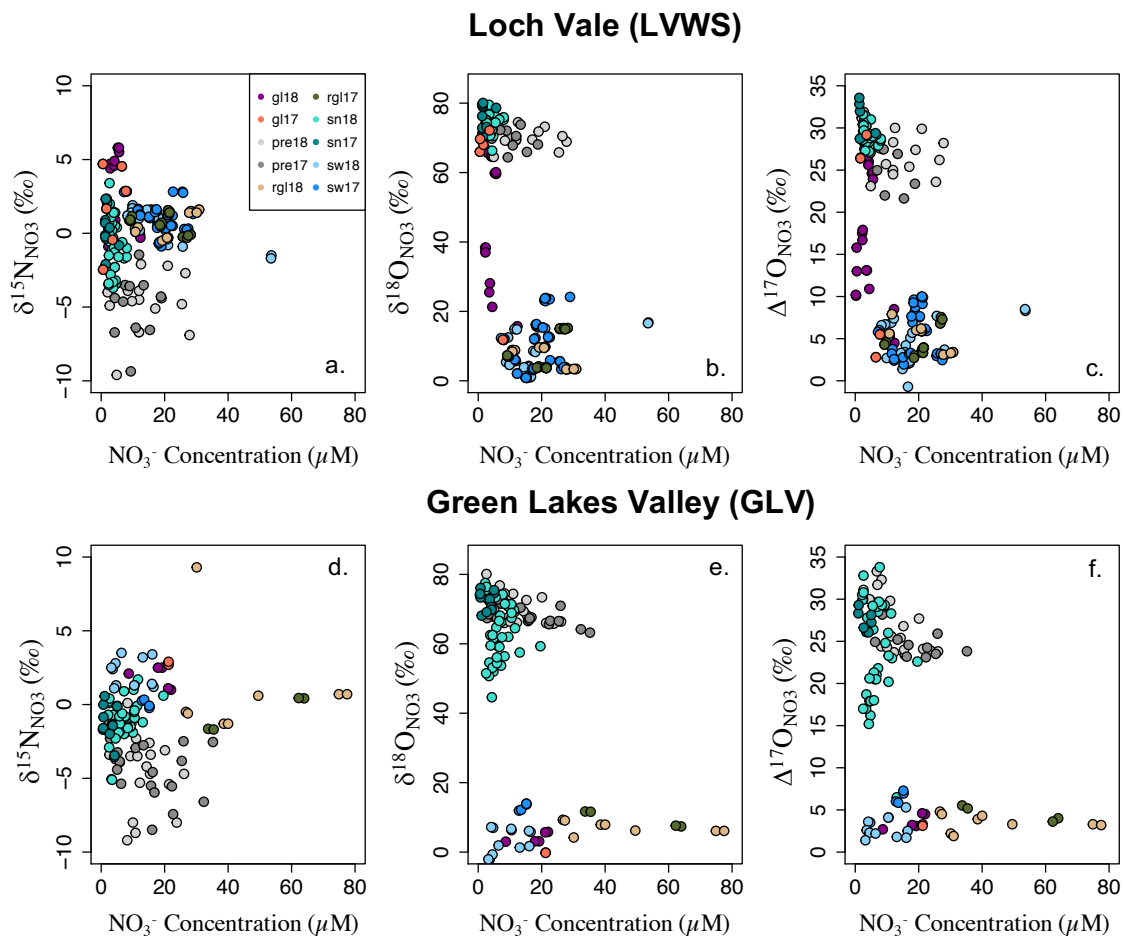


Figure S3: Isotopic composition of $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$ vs. NO_3^- concentration (μM or $\mu\text{mol L}^{-1}$) for LVWS (a-c) and GLV (d-f). End-members represented included glacial ice/meltwater (gl), rain (pre), rock glacier meltwater (rgl), snow (sn), and surface water (sw) from both 2017 and 2018 field campaigns.

Loch Vale Watershed	NO ₃ ⁻ (μM)	n	δ ¹⁵ N _{NO3} (‰)	n	δ ¹⁸ O _{NO3} (‰)	n	Δ ¹⁷ O _{NO3} (‰)	n	NH ₄ ⁺ (μM)	n
Rain. 2017	5.2 to 19.4	13	-8.0 to -3.0	13	65.2 to 73.8	13	21.8 to 27.6	9	8.1 to 23.7	13
	12.8 ± 5.1		-4.6 ± 2.0		70.6 ± 3.6		25.2 ± 2.5		16.9 ± 6.0	
Rain. 2018	5.6 to 16.3	16	-5.8 to -3.0	16	68.6 to 76.6	16	24.7 to 30.6	16	9.7 to 31.6	16
	10.6 ± 4.5		-4.6 ± 1.1		71.2 ± 3.4		27.3 ± 2.6		16.8 ± 9.2	
Snowpack 2017	1.3 to 6.4	13	-2.1 to 2.3	12	70.5 to 80.1	12	26.5 to 33.6	6	0 to 2.4	13
	2.3 ± 1.7		0.6 ± 1.4		74.8 ± 3.6		30.5 ± 2.7		1.2 ± 0.8	
Snowpack 2018	0 to 8.1	48	-3.7 to 3.4	38	66.3 to 79.5	38	27.2 to 31.6	41	0.6 to 8.6	48
	3.4 ± 1.7		-0.8 ± 1.9		73.9 ± 2.4		29.3 ± 1.0		3.3 ± 2.0	
Glacial Ice 2017	0.2 to 6.5	9	-2.5 to 4.7	5	-10.6 to 72.1	5	2.8 to 33.2	5	0.2 to 29.8	9
	1.6 ± 2.1		1.6 ± 3.1		53.0 ± 35.6		23.8 ± 12.0		8.0 ± 9.2	
Glacial Ice 2018	0.2 to 5.6	18	4.4 to 5.8	9	59.6 to 66.5	9	10.1 to 28.4	14	0.3 to 14.0	18
	2.4 ± 2.1		5.0 ± 0.6		63.6 ± 2.9		21.3 ± 7.0		5.7 ± 5.3	
Glacial Meltwater 2017	7.6 to 9.7	5	0.8 to 2.9	5	7.0 to 11.8	5	4.3 to 5.6	4	0.2 to 0.4	2
	8.6 ± 0.9		1.7 ± 1.0		9.0 ± 2.6		5.0 ± 0.7		0.3 ± 0.1	
Glacial Meltwater 2018	2.2 to 12.3	7	-0.9 to 1.5	8	15.6 to 38.4	6	4.5 to 17.9	6	2.4 to 6.7	7
	5.6 ± 4.6		0.2 ± 0.6		27.5 ± 5.9		12.8 ± 3.7		4.7 ± 1.5	
RG Meltwater 2017	18.1 to 28.2	18	-0.7 to 1.6	16	3.7 to 16.3	16	2.7 to 9.6	11	0 to 10.1	18
	22.3 ± 3.6		0.3 ± 0.8		10.4 ± 6.0		5.6 ± 2.6		0.9 ± 2.4	
RG Meltwater 2018	10.8 to 53.6	11	-1.7 to 1.6	11	3.3 to 16.8	11	3.1 to 8.5	11	0 to 5.2	11
	27.3 ± 15.3		0.0 ± 1.1		9.0 ± 4.7		5.8 ± 1.9		0.6 ± 1.8	
Surface Water 2017	7.2 to 28.9	23	0.2 to 2.9	20	3.4 to 24.2	20	3.2 to 10.0	16	0.3 to 1.5	23
	17.6 ± 5.7		1.1 ± 0.8		14.5 ± 7.0		6.6 ± 2.4		0.7 ± 0.4	
Surface Water 2018	8.3 to 28.4	24	-1.7 to 1.9	26	0.9 to 16.8	26	-0.7 to 8.5	25	0 to 2.6	24
	18.9 ± 12.0		0.8 ± 1.0		7.6 ± 5.4		4.5 ± 2.4		0.6 ± 0.8	

Table S1: Range and mean ± standard deviation for each isotope (δ¹⁵N_{NO3}, δ¹⁸O_{NO3}, Δ¹⁷O_{NO3}) and nutrient concentration (NO₃⁻ and NH₄⁺), where n = number of samples analyzed. Samples were collected during the summers of 2017-2018 in Loch Vale Watershed in Rocky Mountain National Park.

Green Lakes Valley	NO ₃ ⁻ (μM)	n	δ ¹⁵ N _{NO3} (‰)	n	δ ¹⁸ O _{NO3} (‰)	n	Δ ¹⁷ O _{NO3} (‰)	n	NH ₄ ⁺ (μM)	n
Rain. 2017	5.8 to 31.8 15.8 ± 12.4	22	-6.3 to -1.7 -3.9±1.9	22	65.2 to 70.9 68.7±2.6	22	23.6 to 28.2 25.3±1.8	10	10.2 to 41.9 25.3 ±13.8	22
Rain 2018	5.6 to 16.3 10.6 ± 4.5	21	-6.8 to -2.0 -4.2±2.0	21	66.7 to 75.5 70.1±3.3	21	23.7 to 31.3 27.3±3.3	21	9.7 to 31.6 16.8 ± 9.2	21
Snowpack 2017	0.5 to 5.1 2.3 ±1.8	12	-3.6 to 0.6 -1.3 ± 1.3	12	68.1 to 76.1 72.6±2.6	12	26.1 to 36.2 28.3±3.2	9	0.2 to 10.1 4.2 ±3.4	12
Snowpack 2018	0.5 to 19.6 5.9 ± 3.9	49	-5.1 to 1.7 -1.1 ± 1.2	38	44.6 to 77.5 63.5 ± 8.4	38	6.5 to 33.8 23.8 ± 6.0	36	0.6 to 19.9 7.1 ± 4.7	49
Glacial Meltwater 2017	21.2 to 21.9 21.5 ±0.4	3	2.7 to 2.9 2.8±0.1	3	-0.2 to -0.1 -0.2±0.1	3	3.1 to 3.3 3.2±0.1	3	-	3
Glacial Meltwater 2018	8.7 to 22.0 17.8 ±5.3	5	1.0 to 2.5 1.8±0.7	5	3.0 to 5.8 4.1 ± 1.4	5	2.7 to 4.6 3.6 ± 0.9	5	0 to 10.7 5.5 ±5.4	5
RG Meltwater 2017	33.7 to 64.0 48.8 ± 16.5	4	-1.7 to 0.4 -0.6±1.2	4	7.4 to 11.7 9.6±2.4	4	3.6 to 5.5 4.6±0.9	4	0 to 0.7 0.3 ± 0.3	4
RG Meltwater 2018	26.7 to 77.5 43.9 ±19.7	9	-1.3 to 9.3 0.9±3.5	8	4.2 to 9.3 7.1 ± 1.8	8	1.9 to 4.8 3.5 ± 1.0	9	0 to 1.6 0.5 ± 0.6	9
Surface Water 2017	12.9 to 15.2 14.4 ±1.1	5	-0.2 to 0.3 0.0±0.3	5	12.0 to 14.0 13.1±1.0	5	5.9 to 7.3 6.5±0.7	4	0.1 to 1.2 0.5 ±0.5	5
Surface Water 2018	3.2 to 16.5 9.1 ±5.3	12	1.1 to 3.5 2.1 ± 0.9	12	-2.1 to 7.2 3.3 ± 3.6	12	1.4 to 5.3 2.9 ± 1.2	12	0 to 2.0 0.8 ± 0.5	12

Table S2: Range and mean ± standard deviation for each isotope (δ¹⁵N_{NO3}, δ¹⁸O_{NO3}, Δ¹⁷O_{NO3}) and nutrient concentration (NO₃⁻ and NH₄⁺), where n = number of samples analyzed. Samples were collected during the summers of 2017-2018 in the Green Lakes Valley.

Study	Watershed	Sample Type	$\delta^{15}\text{N}_{\text{NO}_3}(\text{‰})$	$\delta^{18}\text{O}_{\text{NO}_3}(\text{‰})$	$\Delta^{17}\text{O}_{\text{NO}_3}(\text{‰})$
Campbell et al., 2002	Loch Vale	Rain	-4 to 1‰	40 to 65‰	-
		Snowpack	0 to 4‰	50 to 70‰	-
		*Snowmelt	0.5‰	40‰	-
		Stream Water	-1 to 2‰	10 to 25‰	-
		*Talus Spring	-3.8 to 1.5‰	10 to 27‰	-
Nanus et al., 2008	Loch Vale	Rain	-3.5‰	71‰	-
		Surface Water	0.5 to 4.6‰	9 to 14‰	-
Nanus et al., 2018		Snowpack	-3.3 to 6.5‰	75 to 82‰	-
		Rain (seasons combined)	-7.6 to 5.5‰	66 to 83‰	-
Clow et al., 2015	RMNP	Rain	-4 to -1‰	66 to 72‰	-
	(e.g., Loch Vale)	Snowpack	-1 to 2‰	43 to 77‰	-
Hafich, 2014	Green Lakes	Rain	-7.5 to -1‰	68 to 75‰	22 to 27‰
	Valley	Snowpack	-1 to 3‰	-	22 to 32‰
		*Snowmelt	-3 to 4.8‰	65 to 75‰	19 to 30‰
		Rock Glacier	-3 to 2.8‰	15 to 18‰	4 to 8‰
		Glacier Meltwater	-3 to 2‰	1 to 45‰	2.5 to 18‰
		Stream Water	-2 to 4.8‰	0 to 20‰	0.5 to 9.5‰
		*Groundwater	2.5 to 4.5‰	-6 to -1‰	1 to 2.5‰
		*Soil Water	-2 to 10.5‰	-3 to 22‰	0.5 to 16‰
		*Subtalus Water	-3.5 to 4‰	0 to 25‰	3.5 to 15‰
		*Snowfield	-3 to 1.5‰	25‰	7 to 12‰

Table S3: Previous isotopic studies in Loch Vale (LVWS) and Green Lakes Valley (GLV) characterizing $\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$ for a variety of sample types. Sample types with an * were not sampled in the current study.

Wynn et al., 2007	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{18}\text{O}_{\text{NO}_3}$	$\Delta^{17}\text{O}_{\text{NO}_3}$
Midtre Lovenbreen Glacial, Svaldbard	(‰)	(‰)	(‰)
Supraglacial streams			
2003 (n=6)	-7.8±2.2	63.1±3.8	-
Subglacial runoff, phase 1			
2002 (n=1)	2.3	-	-
2003 (n=4)	4.0±5.5	-	-
Subglacial runoff phase 2			
2002 (n=4)	-4.5±1.3	-	-
2003 (n=4)	-5.5±1.1	20.3±6.1	-
This study	$\delta^{15}\text{N}_{\text{NO}_3}$	$\delta^{18}\text{O}_{\text{NO}_3}$	$\Delta^{17}\text{O}_{\text{NO}_3}$
Loch Vale and Green Lakes Valley, CO	(‰)	(‰)	(‰)
Glacial Ice (Loch Vale)			
2017 (n= 5, 5, 5, 4)	1.6±3.1	53.0±35.6	23.8±12.0
2018 (n= 9,9,14,9)	5.0±0.6	63.6 ± 2.9	21.3 ± 7.0
Lateral glacial stream (Loch Vale)			
2018 (n=3, *n=1)	-0.5±0.4	37.9±0.8	17.3±0.6
Glacial meltwater (Loch Vale)			
2017 (n= 5,5,4)	1.7±1.0	9.0±2.6	5.0 ± 0.7
2018 (n=6,6,6,4)	0.2 ± 0.6	27.5 ± 5.9	12.8 ± 3.7
Glacial meltwater (Green Lakes Valley)			
2017 (n= 3,3,3)	2.8±0.1	-0.2±0.1	3.2±0.1
2018 (n = 5,5,5,3)	1.8±0.7	4.1 ± 1.4	3.6 ± 0.9

Table S4: Comparison of glacial isotopic composition of nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$, $\delta^{18}\text{O}_{\text{NO}_3}$, $\Delta^{17}\text{O}_{\text{NO}_3}$) in this study with Wynn et al., 2007. Mean ±standard deviation are reported for each sample type by year and study system.

Year	Early Summer	Late Summer	Sample Type
2017	14.4 μM (23.9%)	-	Surface Water
	34.6 μM (19.6%)	63.1 μM (13.9%)	Rock Glacier
2018	13.4 μM (17.4%)	7.8 μM (8.6%)	Surface Water
	34.9 μM (13.4 %)	67.3 μM (14.2%)	Rock Glacier

Table S5: Average NO_3^- $\mu\text{moles L}^{-1}$ (μM) and percent atmospheric nitrate (%) for Green Lakes Valley high elevation lakes and rock glacier inflows organized by early and late summer in 2017-2018.

Appendix One

Effluent gas flux characterization during pyrolysis of chicken manure

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Published: *Sustainable Chemistry & Engineering*. (2017), [doi: 10.1021/acssuschemeng.7b00815](https://doi.org/10.1021/acssuschemeng.7b00815)

Abstract

Pyrolysis is a viable option for the production of renewable energy and agricultural resources from diverted organic waste streams. This high temperature thermochemical process yields material with beneficial reuses, including bio-oil and biochar. Gaseous forms of carbon (C) and nitrogen (N) are also emitted during pyrolysis. The effluent mass emission rates from pyrolysis are not well characterized, thus limiting proper evaluation of the environmental benefits or costs of pyrolysis products. We present the first comprehensive suite of C and N mass emission rate measurements of a biomass pyrolysis process that uses chicken manure as the feedstock to produce biochar and bio-oil. Two chicken manure fast pyrolysis experiments were conducted at controlled temperature ranges of 450 - 485 °C and 550 - 585 °C. Mass emission rates of nitrous oxide (N_2O), nitric oxide (NO), carbon monoxide (CO), carbon dioxide (CO_2), methane (CH_4) and ammonia (NH_3) were measured using trace gas analyzers. Based on the system mass balance, 23-25% of the total mass of the manure feedstock was emitted as gas, while 52-55% and 23% were converted to bio-oil and biochar, respectively. CO_2 and NH_3 were the dominant gaseous species by mass, accounting for 58 - 65% of total C mass emitted and 99% of total N mass emitted, respectively. Temperature variations within the two set of temperature ranges had a perfunctory effect on bio-oil production and gaseous emissions, but the higher temperature range process produced more bio-oil and slightly less emissions. However, a larger effect on the relative amounts of CO and CO_2 produced were observed between the different temperature regimes. These results have important implications for greenhouse gas and reactive nitrogen life cycle assessments of biochar and bio-oil.

1. Introduction

Biochar is a by-product of pyrolysis (anaerobic) or gasification (aerobic) thermal decomposition processes of organic matter. While pyrolysis and gasification technologies have been in use for decades, these processes have received greater attention in recent decades as bio-oil and/or syngas could be used as a renewable biofuel alternative to fossil fuels and biochar soil amendments could reduce the negative consequences of fertilizer for air quality, climate change and aquatic and terrestrial acidification.^{1,2} Biochar has revealed promise in promoting carbon (C) sequestration,³⁻⁵ minimizing greenhouse gas emissions,^{6,7} increasing soil fertility⁸ and crop productivity,^{6,8} increasing water availability in drought-prone conditions,⁹ increasing bioavailability of adsorbed ammonia¹⁰ and preventing nutrient leaching.¹¹ In addition, biochar products minimize volume and mass of the original feedstock,¹² reducing transportation costs of organic waste resources.

Biochar has been produced from many organic feedstocks, including grasses, woody biomass and manures.^{3,12,13} Manure-based biochar use could mitigate greenhouse gas emissions and reactive nitrogen (Nr) pollution to air and water from concentrated animal feeding operations.¹⁴ Fast pyrolysis of organic feedstocks for biochar produces bioenergy resources (i.e. bio-oil) that can displace a portion of fossil fuel-based energy, and biochar as a co-product.¹⁵

While evidence for pyrolysis product benefits for agriculture and renewable energy is mounting, a key uncertainty remains in the assessment of their biogeochemical life cycle impacts. The magnitude and forms of mass emission rates during pyrolysis of organic feedstocks to produce bio-renewable products are not well quantified. Carbon gases emitted during pyrolysis include major greenhouse gases: carbon dioxide (CO₂), methane (CH₄), and air pollutant carbon monoxide (CO). Nitrogen gases emitted during pyrolysis include non-reactive

dinitrogen (N_2), as well as several forms of Nr such as ammonia (NH_3), nitrous oxide (N_2O), and nitrogen oxides (NO_x). Reactive forms of N have cascading impacts on environmental systems and human health.^{2,16} Therefore, it is critical to understand the potential unintended losses of C and N during the production of biochar and bio-oil in order to assess the net benefits of its utility for mitigating climate change and/or reactive N pollution, particularly from high N-content feeds such as chicken manure.¹⁷ In this study, we measure total C and Nr mass emission rates during fast pyrolysis of chicken manure to quantify and characterize the dominant N and C species emitted during the pyrolysis process. Experiments were conducted at two temperature ranges (450 – 485 °C and 550 – 585 °C) to determine the effect of temperature as a proximal control on mass emission rates. This work constitutes some of the first C and Nr mass emission rates during the pyrolysis of chicken manure to produce bio-oil and biochar, which can be compared with future experiments under different conditions. These measurements provide a foundation for future C and N life cycle analyses, which can address applicability of this technology at large and small scales and analyze the cost benefit of reducing manure in pollution hotspots.

2. Experimental (Materials and Methods)

2.1 Experimental design

Effluent gas fluxes of reactive N species (NO and NH_3), greenhouse gases (N_2O , CH_4 and CO_2), and carbon monoxide (CO) were measured during the pyrolysis of chicken manure. Chicken manure was collected from conventional broiler poultry production houses raising the Cobb chicken breed with unlimited grain feed rations according to standard industry practices (Frye Poultry, West Virginia). Manure consisted of fresh scrapings of house floors and included chicken manure and sawdust.¹⁸ All manure was dried, sieved and homogenized to 2 mm grain

size for pyrolysis efficiency¹³ (see sections: Exp 1: Low Temperature (450 - 485 °C and Exp 2: High Temperature (545 – 585 °C) for more details). Small particles are needed for rapid heat transfer to the entire biomass material for fast pyrolysis.¹⁹

Manure was pyrolyzed using USDA's bubbling fluidized bed pyrolysis development unit (PDU) located at the Eastern Regional Research Center (Wyndmoor, PA). The reactor bed consisted of a 7.6 cm diameter pipe filled to static bed depth of 20 cm sand, heated electrically to the temperatures mentioned above. Manure was fed by an auger directly into a bubbling sand bed fluidized with nitrogen. The entrained pyrolysis solids and vapors travel to a cyclone separator where the biochar is removed from the vapor stream and collected. The vapors continue onto a train of four chilled condensers in series to remove condensable vapors followed by two electrostatic precipitators used for capturing aerosols. This pyrolyzer is capable of producing bio-oil under variable temperature conditions, with the additional co-products of this process including biochar and non-condensable gas (NCG). A detailed description of the bench-scale pyrolysis instrument used, evidence of fast pyrolysis efficiency using 2 mm grain size and controls on exhaust flow rate is available in Boateng et al. 2007.¹³

Two experiments were conducted starting with a fully cleaned pyrolyzer. Experimental conditions for each run are provided in Table 1. Temperature was set at 450 – 485 °C (low temp, exp 1) and at 550 – 585 °C (high temp, exp 2) and constraints were placed on feed rate, N₂ dilution and flow rate. A TGA study shows that chicken manure experiences maximum loss at similar temperatures to most lignocellulosic biomass.²⁰ It has also been well established that temperatures between 450 °C and 600 °C maximize bio-oil production for biomass,¹⁹ and the temperatures chosen provide two regimes within this range to evaluate emissions associated with

fast pyrolysis of chicken manure. However, future work should consider testing the effect of other temperature ranges, as well as repeatability on gas effluent during this process.

Experimental Constraints	Exp 1	Exp 2
Temp Range (°C)	450-485	546-588
Run Time (minutes)	110	159
Average Feed Rate (kg/h)	2.78	2.11
Average N ₂ Dilution (L/min)	80	93
Manure Moisture (%)	27.21	27.51
Product Masses Post Pyrolysis	Exp 1	Exp 2
Biochar (kg)	0.825	1.14
Bio-oil (kg)	0.378	0.838
Gas (kg)	0.711	1.10
Biochar (% composition by mass)	23.3	23.2
Bio-oil (% composition by mass)	51.9	54.6
Gas (% composition by mass)	25.0	23.3

Table 1: Experimental conditions, product masses and percent composition by mass from pyrolysis of chicken manure.

2.2 Gas Measurements

Trace gas concentration measurements were performed using an open-path quantum cascade laser for NH₃,²¹ cavity ring-down spectroscopy for CH₄ and CO₂ (Picarro, Model G2301), ICOS (integrated cavity output spectroscopy) for CO and N₂O (Los Gatos Research, Model 907-0014); and chemiluminescence for NO and NO_x (ThermoFisher Scientific, Model 42i analyzer). The NH₃ sensor provides 0.1 sec resolution concentration measurements and retrievals were calibrated before and after experiments from 10 ppbv (ambient NH₃) to 9 ppmv NH₃ to within 0.20 ppbv $\pm$ 20% using methods described in Miller et al. (2014).²¹ Linear calibration curve fitting was applied up to 2 ppmv and logarithmic fitting from 2-9 ppmv NH₃. Above 9 ppmv NH₃, concentrations were estimated using offline spectral fitting of direct absorption spectra up to a maximum of 12 ppmv NH₃ (assuming non-linear absorption with concentration in

this range as optical saturation limits were approached).²¹ CH₄, N₂O and CO were measured at 1 sec and CO₂ at 5 sec resolution. The precision of these measurements is discussed in section *Mass Balance and Gas Flux Uncertainties*. The NO_x Analyzer was used for 8 sec resolution NO and NO_x measurements and were calibrated to within $\pm 5\%$ accuracy using a dilution calibrator (ThermoFisher Scientific, Model 146i): using 50.2 ppmv NO in N₂ and 10 ppmv NO₂ in N₂ gas standards diluted with zero air (ThermoFisher Scientific, Model 111) over a range from 0-500 ppbv NO_x. All data was recorded in real-time by a laptop computer.

All gases were captured within a static flux chamber designed to enclose the NH₃ sensor and a three-stage measurement process (2-3 minutes per stage) was conducted as follows: (1) NH₃ desorption flux from the chamber surface was monitored before introducing the flux to the chamber, (2) fluxes for all gaseous species were measured, and (3) NH₃ adsorption onto the chamber walls was monitored while flux to the chamber stopped. A slipstream of gas exhaust (typical slipstream flow rate was $\sim 150 \text{ mL min}^{-1}$) was piped from the pyrolyzer to a 6.5 L static flux chamber using one PTFE Teflon tubing (1/8" ID x 1/4" OD) where all gases could be measured simultaneously at the same inlet. Two PTFE particle filters (1.0 micron, 47 mm) were used to remove particulate matter from the exhaust slipstream prior to entering the chamber. The total exhaust flow rates were 80 or 93 L min⁻¹ during experiments one and two, respectively. The flow rate was increased for the second experiment in an effort to dilute the analyte gases (particularly NH₃) to provide a better concentration range for the NH₃ laser. Both flow rates provided residence times appropriate for fast pyrolysis and were not expected to have a significant effect on the chemical reactions (although examining the impact of flow rate variability on gas fluxes could be considered in future research). During the flux measurements, a microcontroller unit (Arduino Uno) recorded temperature, pressure and relative humidity

simultaneously within the flux chamber with a digital relative humidity and temperature sensor (HYT 271, IST Innovative Sensor Technology, accuracy $\pm 1.8\%$ RH and ± 0.2 C) as well as a digital pressure sensor (MS5803-01BA, Measurement Specialties Inc., accuracy ± 0.5 hPa). Exhaust temperature ranged from 23.7 - 29.3 °C, pressure was steady at 999.48 ± 1 hPa and relative humidity ranged from 25 – 59%.

2.3 Exp 1: Low Temperature (450 - 485 °C)

The first batch of manure was sieved and homogenized to 2 mm grain size using a seven-bladed Wiley Mill (Thomas Scientific, Model 4, Swedesboro, NJ). All manure was sealed and stored at room temperature until pyrolysis measurements could take place (< 1 month since collection). Storage times were kept to a minimum to reduce potential ammonia volatilization and changes in N content. Manure was kept tightly sealed and stored in buckets lined with compostable bags. There were no obvious signs of decomposition during storage. After the fluidized bed was heated to 450 – 485 °C, manure was incorporated into the feedstock funnel at a constant rate and the primary gas stream was diluted with N₂ (Table 1). Over 110 minutes, 10 flux measurements (6 – 8 minutes per chamber measurement) were taken of all 6 gaseous species. The first 3 measurements reflect background levels prior to any chicken manure feedstock addition, which occurred at around 40 minutes.

2.4 Exp 2: High Temperature (545 – 585 °C)

The second batch of manure was oven-dried at 60 °C overnight and then homogenized using the Wiley Mill to 2 mm grain size to produce a drier feedstock. A drier manure is less likely to slow pyrolysis reactions and will make the process more efficient. Fluidized bed

temperatures were set from 550 – 585 °C using drier manure to conduct mass emission rate measurements under a steady feed rate and N₂ dilution (Table 1). Over 159 minutes, 18 flux measurements were (6 - 8 minutes per chamber measurement) were taken of all 6 gaseous species with the first 4 measurements reflecting background levels before the addition of feedstock at around 40 minutes.

Subsamples of manure, biochar and bio-oil were weighed, oven-dried at 60 °C overnight and reweighed to calculate the moisture content for each experiment. These samples were analyzed for percent total organic C and total N using Elemental Analysis (Thermo EA1112 CHNS/O Analyzer), which were used for mass balance calculations. The uncertainties of C and N content estimates from elemental analysis were quantified by the standard deviation (1σ) between multiple replicate samples for each pyrolysis co-product as well as the original biomass for each experimental batch with mean %C or %N ± 1σ reported in Table S1.

2.5 Mass Balance Approach

Exhaust losses were also calculated using a mass balance approach. With known initial masses of manure and final masses of biochar and bio-oil, initial and final masses of C and N were calculated using the following equations:

$$I_{C\ Mass} = I_{dry\ manure} * \left(\frac{P_{\%C\ manure}}{100} \right) \quad (1)$$

$$F_{C\ Mass} = F_{biochar\ mass} * \left(\frac{P_{\%C\ biochar}}{100} \right), \quad (2)$$

where $I_{C\ Mass}$ is the initial manure C mass, $I_{dry\ manure}$ is the initial mass of manure prior to pyrolysis, $P_{\%C\ manure}$ is the %C of manure, $F_{C\ Mass}$ is the final biochar C mass, $F_{biochar\ mass}$ is the final mass of biochar post pyrolysis and $P_{\%C\ biochar}$ is %C of the biochar. These were used to calculate losses (from the system) in total C mass (3) and total N mass (4). Total mass lost is the

gas estimated from the difference in N₂ input – N₂ output from the GC used at the USDA facility, while C and N mass loss are calculated by difference from that contained in the bio-oil and biochar.

$$\text{Total C Mass Lost} = \text{Initial Manure C} - \text{Final Biochar C} - \text{Final Bio-oil C} \quad (3)$$

$$\text{Total N Mass Lost} = \text{Initial Manure N} - \text{Final Biochar N} - \text{Final Bio-oil Mass} \quad (4)$$

Uncertainty in mass loss differences was calculated using error propagation for mass balance using equation (5), where δa , δb and δc is the uncertainty associated with C or N for manure, biochar and bio-oil, respectively and results are shown in Table S2.

$$\delta N \text{ or } \delta C = \sqrt{\delta a^2 + \delta b^2 + \delta c^2} \quad (5)$$

2.6 Effluent Gas Flux Approach

Gas flux measurements were used to calculate total mass emitted for each C and N species. First, all gas analyzer timestamps were synchronized to the laptop computer time before recording data. Each flux measurement period was picked out of the time series during rising gas concentrations. A single gas (NO) was selected to ensure appropriate temporal alignment of the fluxes for synchronous flux calculations. Least squares linear regression was used to estimate the slope of each concentration time series and assumed that the slope was constant during the 2 - 3 minutes of each chamber measurement. The mass emission rate of each C and N species in kilograms N or C per minute (kg min⁻¹) was determined using:

$$E = \frac{VP}{RT} * \frac{dC}{dt} * mw, \quad (6)$$

where V was the fixed volume of the flux chamber (6.5 L or 0.0065 m³), P and T were measured pressure (1 atm) and temperature (25 °C), $\frac{dC}{dt}$ was defined as the slope of the regression fit (ppmv min⁻¹) and mw is the molecular weight of the corresponding element (C or N) in g mol⁻¹.

The adsorption and desorption flux monitoring was conducted to evaluate how the surface affinity of NH_3 affected the chamber measurements. For a majority of flux measurements, within one minute of beginning the flux stage ammonia inside the chamber reached levels of optical absorption saturation above which concentrations could not be retrieved and flux corrections could not be performed. However, based on measurements below these limits, NH_3 concentrations (>10 ppmv) during flux measurements were higher than the wall saturation point and adsorption was the dominant process for NH_3 losses. For all gases, we scaled the flux chamber measurements to the total exhaust stream using the ratio of total exhaust to slipstream flow. During experiment one, the slipstream flow rate was stable at 150 mL min^{-1} . However, flow was more difficult to stabilize during the second experiment due to issues with filter clogging in the line, but was typically 309 mL min^{-1} . Periodic flow measurements with a flow meter (Dry Cal Pro, MesaLabs, Model Defender 520) were linearly interpolated to estimate flows across the emission measurement period (Table S3). The total emission mass uncertainties were quantified. Each flux measurement uncertainty was estimated using the root mean squared error (RMSE) of the regression slope as in Laville et al. (2011)²² (Table S4). The mass emission rate time series was integrated across the experimental period to obtain total mass (kg N or C) of each species emitted. The total mass of N and C gases were compared with the mass balance results to assess differences between both methods of calculating gaseous loss. Uncertainty in mass loss differences were calculated using the average gas emission rate and the experimental time duration to characterize total mass emitted (Table S5), at which point mass uncertainty was calculated by taking into account the instrumental error (δF) to calculate the δM for each gas measured (Table S5). Total N and C error was propagated using equation (5), where δa , δb and δc were uncertainties associated with each species (Table S5). After propagating the elemental

error separately by approach (MB vs GF), the total error for C and N between methods was calculated using the same approach (Table S6). Differences might reveal whether the gases we measured account for most of the gaseous losses or if our measurements are missing gases such as organic N, other NO_y, particulate ammonium nitrate, hydrocarbons or organic particulates not removed by precipitators. All data processing was performed using a commercial software package (MATLAB 8.6, The MathWorks Inc., Natick, MA, R2015b).²³

3. Results

Pyrolysis product masses and experimental conditions shown in Table 1 show that differences between experimental conditions resulted in similar outcomes for the redistribution of mass by product. As shown in Table 1, the percent composition by dry mass of each product was 23% (biochar), 25% (gas) and 52% (bio-oil) for experiment 1 and 23% (biochar), 23% (gas) and 55% (bio-oil) for experiment 2. The manure moisture contents were ~27% in both experimental batches (Table 1), which suggests that the pre-drying treatment for the second batch was either ineffective at creating a drier manure or the initial manure moisture was greater than 27%. While C and N gases were the focus of this study, ash content and other elements (hydrogen, oxygen and sulfur) were characterized on a dry-basis mass (see supplement Table S1).

3.1 C & N Emissions by Mass Balance

C and N dry-basis, percent composition of each co-product had lower %C and %N contents for manure, biochar and bio-oil in experiment one compared to experiment two (Table S1). Standard deviations for %C and %N were 0.84% or less for all measurements (Table S1).

Mass balance calculations suggest that 0.71 kg (27.6%) of the total initial manure mass was emitted as mostly gas during the pyrolysis process, but small amounts of particulate matter, uncondensed reaction water and uncondensed volatile organics are also emitted. There was a 0.562 kg mass loss as some form of C (51.1% of input C), while there was only a 0.052 kg mass loss as some form of Nr (52% of input N) (Table 2). Mass balance calculations indicate that 1.104 kg (23.3%) of total mass was lost during pyrolysis in experiment two (Table 2). Of this total mass, there was a 0.623 kg (36.6%) emission of C and a 0.059 kg (36.2%) emission of N (Table 2).

Mass Loss Calculations from Mass Balance	Exp 1		Exp 2	
	kg	%	kg	%
Initial manure mass, dry (kg)	2.574	-	4.075	-
Initial manure C mass (kg)	1.098	-	1.720	-
Initial manure N mas (kg)	0.099	-	0.165	-
Final biochar mass, dry (kg)	0.825	-	1.140	-
Final biochar C mass (kg)	0.274	24.9%	0.486	28.2%
Final biochar N mass (kg)	0.017	17.1%	0.035	21.1%
Final bio-oil mass, dry (kg)	0.378	-	0.838	-
Final bio-oil C mass (kg)	0.263	23.9%	0.611	35.5%
Final bio-oil N mass (kg)	0.028	28.8%	0.071	23.0%
Total mass lost (kg)	0.711	-	1.104	-
Total C mass lost (kg)	0.562	51.1%	0.623	36.2%
Total N mass lost (kg)	0.052	52.5%	0.059	35.7%
Total C + N mass lost (kg)	0.614	51.3%	0.682	36.1%

Table 2: Mass balance calculations illustrating mass losses using biochar, manure and bio-oil components for experiments 1 and 2.

3.2 C & N Emission by Effluent Gas Flux

During experiment one, produced CO₂ and NH₃ were the highest source of gas emissions (Tables 3 and S7) for C and N species measured respectively, which equates to 66% of total C emissions as CO₂ and ~ 99% of total N as NH₃ gaseous emissions measured (Table 3). The next

prominent emissions were as CO and CH₄ for carbon and NO and N₂O for nitrogen (Tables 3 and S7). The total C and N mass emitted during pyrolysis (of what we measured in the gas phase) was 0.089 kg (Table 3). Of this mass, 0.080 kg was emitted as total C and 0.010 kg was emitted as total N (Table 3). Carbon species (CO₂, CH₄ and CO) accounted for 66%, 5.2% and 29% of the total C emissions (Figure 1; Table 3). Nitrogen species (NO, N₂O and NH₃) comprised 0.34%, 0.08% and 99% of total N emissions (Figure 1; Table 3). These compositions are further illustrated by mole percent for entire gas emissions for both C and N (Figure S1, panels a, c).

Gas	Total Mass (kg C or N)	% C or N mass of measured gases	
CO ₂ -C	5.2E-02	66	
CH ₄ -C	4.2E-03	5.2	
CO-C	2.3E-02	29	
Total C	8.0 E-02	100.2	
NO-N	3.3E-05	0.34	
N ₂ O-N	7.4E-06	0.08	
NH ₃ -N	9.7E-03	99	
Total N	9.7E-03	99.42	
Ratio of slipstream: Full flow		85L min ⁻¹ :150mL min ⁻¹	566.667
C+N mass lost		8.9E-02	
Total C mass lost		8.0E-02	
Total N mass lost		9.7E-03	
Missing C (kg)		4.8E-01	
Missing N (kg)		4.2E-02	

Table 3: Total masses of each element by species emitted (e.g C content of CO₂ emitted) for experiment 1 over 110 minutes scaled by the ratio of slipstream:full exhaust flow. Gas emissions were broken down by the percent measured of the total composition using total mass emitted as kg C or kg N. See Table S4 for experiment 2 results. C+N mass lost is the cumulative gas emissions.

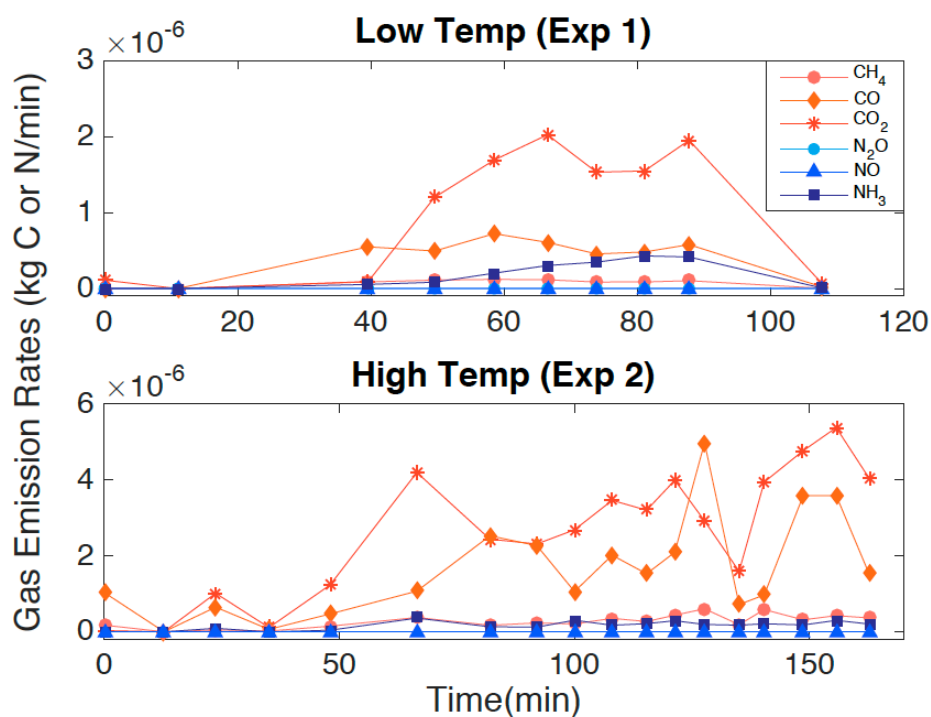


Figure 1: Gas flux time series during Low Temp (exp 1) and High Temp (exp 2) of each gas species in kg C or N/min. N_2O is not present in the second experiment because measurements indicated that fluxes were near zero. Note the change in scales for experiment 2. Feedstock was introduced at 40 minutes after initial background gas levels were recorded.

Similar patterns were observed in gaseous abundance and composition of the pyrolysis exhaust during both experiments where CO_2 and NH_3 had the highest emissions (Tables S7 and 4). Using the area of the total mass emitted, the total C and N mass emissions were 0.21 kg during experiment two (Table 4). Of this mass, 0.20 kg was lost as total C and 0.0078 kg was lost as total N (Table 4). The relative variations of all gas emission rates were very similar between experiments and total mass emitted was higher for the longer second experiment (Figure 1; Tables S7, 4). CO_2 , CH_4 and CO accounted for 58%, 5.9% and 36% of the C emissions measured (Figure S2; Table 4). N_2O , NO and NH_3 comprised 0%, 0.68% and 99% of total N emissions measured (Figure S2; Table 4). Elemental composition is represented for both C and N in mole percent, which further demonstrates that CO_2 , CO and NH_3 are the dominant gaseous

components for C and N (Figure S1, panels b, d). The concentrations of N₂O were near ambient atmospheric concentrations in the flux chamber during experiment 2, therefore are not currently incorporated into the emission calculations for the second experiment. The percent differences in emission rates between the first (lower temperature) and second (higher temperature) experiments were -7.3% for CO₂, + 0.65% CH₄, and + 6.7% for CO while NO changed by + 0.33% and -0.32% for NH₃.

Gas	Total Mass (kg C or N)	% C or N mass of measured gases	
CO ₂ -C	1.2E-01	58	
CH ₄ -C	1.2E-02	5.9	
CO-C	7.3E-02	36	
Total C	2.0E-01	99.68	
NO-N	5.3E-05	0.68	
NH ₃ -N	7.8E-03	99	
Total N	7.8E-03	99.42	
Ratio of slipstream: average full flow		93L min ⁻¹ : 309 mL min ⁻¹	301.113
C+N mass lost		2.1E-01	
Total C mass lost (kg)		2.0E-01	
Total N mass lost (kg)		7.8E-03	
Missing C (kg)		4.2E-01	
Missing N (kg)		5.2E-02	

Table 4: Masses of each element by species emissions (e.g C content for CO₂) for experiment 2. The calculated ratio of slipstream:full exhaust flow was used in tandem with the integrated area of each gaseous element to calculate the total mass emitted during the experimental period by gas and element. Slipstream flow rate was ~309 mL min⁻¹ Gas emissions were broken down by the percent measured of the total composition using total mass emitted as kg C or kg N. C+N mass lost is the cumulative gas emissions.

3.3 Mass Balance vs Effluent Gas Flux

The gas flux approach produced estimates of total mass losses, which were a factor of 3-6 (for carbon) and 5-8 (for nitrogen) lower than those calculated from the mass balance approach.

Mass loss differences/deficits for C+N mass, total C mass and total N mass between the two methods are as follows: 0.525 (43.8% of total input), 0.482 (43.9%) and 0.042 kg (42.4%) for low temperature conditions (Figure 2, Table S8) while mass loss differences appear much lower during high temperature conditions of experiment 2 (0.470 kg or 24.9%, 0.419 or 24.3%, and 0.051 kg or 30.9%) for C+N mass, total C and total N – Figure 2, Table S8). The discrepancies between each method potentially suggest that the gas flux method underestimates mass loss compared to the mass balance approach and/or that the mass balance approach is overestimating the emissions. It is likely that the gas flux measurements are an underestimation possibly due to concentration measurement uncertainties, slipstream flow stability, particulates or additional C and N sources unaccounted for by our measurements, particularly with respect to C species, are discussed in the next section. On the other hand, the mass balance emissions may be overestimated because organic bio-oil that deposits within the pyrolysis system is unaccounted for as emissions. This source of error in the mass balance is lower in experiment two likely because of the large amount of manure run, which makes the percentage amount of bio-oil deposited on surfaces within the pyrolysis system lower. Although each approach had sources of error associated with them, the combination of the two approaches provides a range of likely emissions from chicken manure pyrolysis at these conditions.

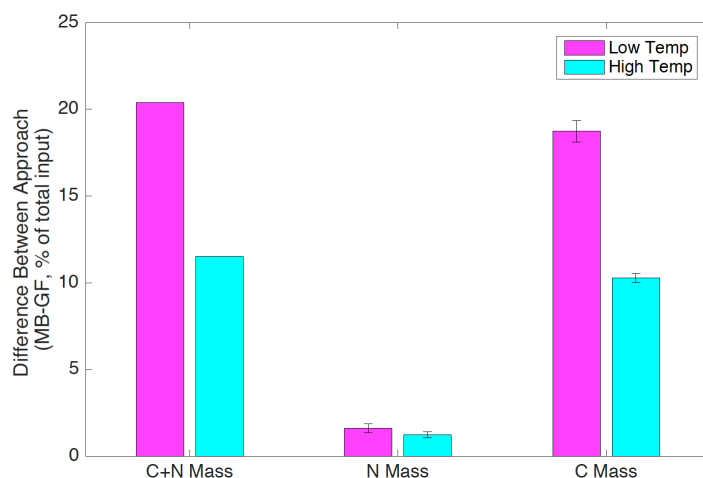


Figure 2: C+N mass, total mass as C and total mass as N differences [% of total dry input; exp 1: 2.574 kg, exp 2: 4.075 kg] between mass balance and gas flux approaches used for each experiment (1 = magenta, 2 = cyan) discussed in section *Mass Balance vs Gas Flux*. Errors were derived from propagation of both mass balance and gas flux approach uncertainties (explained in section *Mass Balance and Gas Flux Uncertainties*).

3.4 Mass Balance and Effluent Gas Flux Uncertainties

Regression slope uncertainties are less than 0.2% during both experiments for all species with the exception of that for NO with 13% median uncertainty for experiment 1 (Table S4). Since regression slope uncertainties ($<0.2\%$) were generally much lower than concentration uncertainties ($\geq 1\%$), linear regression slope fitting does not introduce significant uncertainties above the concentration uncertainty levels for the majority of our gas measurements (Table S5). Therefore, we estimate gas flux uncertainties using the concentration uncertainties in Table S5 and average flux values across the experimental period. The error propagation for total N and C gas emissions results in $2.3\text{E-}03$ and $1.6\text{E-}03$ kg N and $6.8\text{E-}04$ and $1.5\text{E-}03$ kg C (for experiments 1 and 2, respectively) uncertainties that cannot account for the 50 - 450 g discrepancies in mass lost derived from the MB and GF methods (Figure 2). Although the 6 - 16

g mass balance approach uncertainties (Tables: 2, S2, S6) dominate the error bars in Figure 2, these errors also cannot account for the mass discrepancies.

We assess the potential for biases due to instability of flow rates and unmeasured C and N gases/particles to account for these discrepancies, especially for C species. The mean slipstream varied by 20% (1σ) during experiment two (Table S3) and could cause underestimation of gas emissions. Altering the gas flow within this uncertainty could make up an estimated missing C mass (~ 0.05 kg), but not all of it (~ 0.36 kg would still be missing). A major source of over-estimation in the mass balance approach for both C and N is bio-oil that condenses in the piping of the pyrolysis system and is therefore not recovered in the collection vessel. Additional sources of error could be C loss as non-condensable particulates we are not incorporating in our calculations or other organic C gases not measured during this study (e.g. ethylene and other light hydrocarbon gases). For N gases, NH_3 emissions are likely underestimated due to adsorption/surface reaction losses. Although the chamber walls provided a sink for NH_3 , the dominant adsorption flux losses in our system were associated with the slipstream tubing/filters at ambient temperature and relative humidity before entering the flux chamber.

4. Discussion

Production conditions and biomass feedstock are important considerations when assessing the overall potential benefits of biochar for soil quality improvements, pyrolysis co-product yield, biomass management and environmental impact. Using fast pyrolysis, our results suggest that both experimental temperature regimes resulted in similar ($\sim 23\%$) biochar yields, while a slightly greater proportion of C and N gas production occurred under low temperatures

(~ 25%) and slightly higher yields in bio-oil occurred at high temperatures (~ 55%) (Table 1, Figures S3, S4). This observation is opposite of what is normally observed under varied temperature conditions in pyrolysis studies, although the differences are very small. Other studies have found larger differences in biochar yield and nutrient properties over larger temperature ranges (300 °C and 600 °C), which also vary by biochar material.^{14,15} The flux measurements presented here will enable progress on constraining optimal pyrolysis conditions while optimizing bio-oil and biochar yield and minimizing noxious gas emissions during manufacturing. Moreover, the release of gases implicated in climate forcing and air quality degradation are important considerations in a life cycle net cost benefit analysis and assessment of the economic feasibility of biochar production from manure.

Technologies that convert poultry manure into bioenergy and soil amendments have the potential to improve the environmental sustainability of poultry waste management from operations at small- and large-scales.²⁴ Poultry production is the fastest growing livestock sector globally.²⁵ Its intensification poses increasing challenges for best treatment and use of manure nutrients. This work has direct implications for future life cycle assessments that can compare net benefits and tradeoffs of manure management practices in hotspots of concentrated chicken manure production, such as the Chesapeake Bay Watershed. Additionally, this work will provide beneficial information to aide in structuring future poultry waste management strategies, such as large-scale biochar production, which will become more important as global poultry production continues to rise. As of 2016, poultry production comprised 115.8 million tons of the 320 million tons of global meat produced annually.²⁶

Of the gaseous components measured, NH₃, CO₂ and CO comprised the largest emissions during each experiment. NH₃ made up ~ 99% of the gaseous Nr emissions (by mass) for both

experiments, while NO and N₂O contributed to less than 5% (Figures S2; Tables 3, 4). At the lower temperature, CO₂ comprised 66% of the total C gaseous content, while CO was 29% (Table 3). These percentages were likely observed due to reducing, anaerobic conditions or possibly hydrocarbon exchange. However, at the higher temperature CO₂ emissions dropped to 58% and CO increased to 36% of total C (Table 4). Interestingly, varying temperature conditions seemed to have an effect on the composition of CO₂ and CO present (Tables: 3, 4). Since pyrolysis is an anaerobic process, the presence of oxidized nitrogen species, such as NO, is somewhat surprising, but perhaps is a function of high moisture, mineral content or reaction with organic oxygen contained in the manure.

Manure wastes have high ammonium content, thus NH₃ volatilization was expected to occur, especially under high heat conditions and is a likely explanation for the abundance of NH₃ measured in total N yield as has been suggested in other work.¹⁷ This result suggests that the process of producing biochar via pyrolysis could emit a substantial amount of NH₃ if manure wastes are utilized as the feedstock and gaseous emissions are not contained or reused, such as in gasification systems. High NH₃ emissions during pyrolysis may also offset potential reactive N mitigation effects of transitioning from raw manure to manure-derived biochar agricultural soil amendments.

In an effort to address the significance of these measurements, conservatively scaling up these NH₃ pyrolysis emissions to an annual production estimate, our gas flux measurements suggest that 1.4×10^{-3} to 2.7×10^{-3} kg NH₃-N would be produced from pyrolysis of one kg of manure (Table S9). Within the Chesapeake Bay Watershed, an estimated 8.64 million kg of excess manure is applied in the region to agricultural soils every year.¹⁴ According to the measurements conducted in this study, if this excess manure was turned into biochar via

pyrolysis prior to land application, as much as 1.2×10^7 kg of NH_3 could be emitted into the air every year increasing the potential impact of atmospheric N deposition without a mechanism to capture the gas exhaust. However, this is potentially a better option than NH_3 volatilization from chicken manure applications onto agricultural land, which can be as high as 20 - 60% of the total N applied and vary with environmental conditions, such as temperature.²⁷ Compared with pyrolysis, emissions for chicken manure applications can be up to an order of magnitude higher ranging from $3.4 \times 10^7 - 1.0 \times 10^8$ kg $\text{NH}_3\text{-N yr}^{-1}$. Within the context of the Chesapeake Bay Watershed and similar regions challenged with the management of concentrated livestock wastes, pyrolysis could minimize water pollution with reduced runoff and improve air quality. However, without a mechanism for capturing or recycling the exhaust the pyrolysis process would still emit 12 – 35% (Table S9) of what would naturally volatilize from land applications of manure. Given this information, it may be more practical to implement a pollution control system on a pyrolysis plant than attempting to control emissions in agricultural fields.

While many studies have tested the utility of wood-based feedstocks for biochar production, recent attention has been focused on the benefits of biochar produced by manures, agricultural wastes and crop biomass, which can be plentiful in the environment and challenging to dispose of properly. Biochar made from chicken manure has been found to enhance N, potassium and phosphorus availability in soils,^{28,29} but may also contribute to reactive N losses to the environment when used as a soil amendment. Field experiments have also shown that crop yields increase and greenhouse gas emissions of species such as N_2O decrease when poultry char is applied to cornfields in sandy and silty-loam agricultural plots.⁶ Additionally, biochar products minimize volume and mass of the original feedstock,¹² which reduces transportation costs allowing for the transport of nutrients within and across watersheds. Enhanced capacity for

transportation would alleviate N pollution hotspots.¹⁴ Further investigations into life cycle cost-benefit analyses^{15,30,31} would allow for estimating regional scale impacts of each of these factors, including production, transportation and utilization in agricultural settings, to assess its realistic applicability and role in mitigating air quality and climate impacts, nutrient leaching and promoting C sequestration.

5. Acknowledgements

This work was made possible by funding provided by the Institute at Brown for Environment and Society (IBES) Seed Grant Program (to MGH and RR) and the Rathmann Family Foundation (to RR). Special thanks to all assistance and support provided by: the United States Department of Agriculture Sustainable Biofuels and Co-Products Division at the Eastern Regional Research Center, Mark Schaffer, Tom Coleman, Craig Einfeldt, Frye Poultry, the Tang Lab at the Marine Biological Laboratory, Elizabeth de la Reguera, Lei Tao, Los Gatos Research, Joseph Orchard, Dave Murray, Ruby Ho and Paul Wotjal. We also thank three anonymous reviewers for constructive feedback on the manuscript.

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7. Supplement to: Effluent gas flux characterization during pyrolysis of chicken manure

Contains: Nine supplementary tables and four supplementary figures.

Element	Experiment 1			Experiment 2		
	Bio-oil	Biochar	Manure	Bio-oil	Biochar	Manure
	Mean $\pm$ 1 σ	Mean $\pm$ 1 σ	Mean $\pm$ 1 σ	Mean $\pm$ 1 σ	Mean $\pm$ 1 σ	Mean $\pm$ 1 σ
Nitrogen (wt%, db)	7.616 $\pm$ 0.001	2.117 $\pm$ 0.022	3.83 $\pm$ 0.245	8.428 $\pm$ 0.001	3.063 $\pm$ 0.058	4.05 $\pm$ 0.186
Carbon (wt%, db)	69.75 $\pm$ 0.005	33.21 $\pm$ 0.844	42.68 $\pm$ 0.552	72.94 $\pm$ 0.005	42.61 $\pm$ 0.254	42.21 $\pm$ 0.253
Hydrogen (wt%, db)	2.740 $\pm$ 0.003	1.995 $\pm$ 0.153	4.679 $\pm$ 0.277	3.144 $\pm$ 0.003	2.099 $\pm$ 0.079	4.729 $\pm$ 0.052
Sulfur (wt%, db)	0.026 $\pm$ 0.000	0.846 $\pm$ 0.038	0.171 $\pm$ 0.117	0.000 $\pm$ 0.000	1.493 $\pm$ 0.365	0.604 $\pm$ 0.029
Ash (wt%, db)	--	55.46 $\pm$ 0.569	12.99 $\pm$ 0.472	--	49.29 $\pm$ 0.213	12.39 $\pm$ 0.036
Oxygen (wt%, db)	14.75 $\pm$ 0.38	6.21 $\pm$ 0.817	30.72 $\pm$ 0.458	11.56 $\pm$ 0.890	1.43 $\pm$ 0.206	31.38 $\pm$ 0.216

Table S1: Mean and standard deviations for nitrogen, carbon, hydrogen and sulfur elemental analysis of manure, bio-oil and biochar for each experiment. These values were used to account for potential error. All values are dry-basis mass (db = dry-basis). Outliers were removed from carbon average and standard deviation calculations for biochar (n=1) and manure (n=2) for experiment 1.

Product	Mass (kg)	%N (Std Dev)	(mass*%(N/100)) ²	Mass (kg)	% C (Std Dev)	(mass*%(C/100)) ²
Manure	2.573	0.2545	4.2905E-05	2.573	0.5516	2.0154E-04
Biooil	0.378	0.00116	1.9226E-11	0.378	0.00488	3.4027E-10
Biochar	0.825	0.0215	3.1461E-08	0.825	0.8441	4.8494E-05
Mass Balance N Error (δ N, kg)			0.0065	Mass Balance C Error (δ C, kg)		
				0.01581		

Table S2a) Mass balance error propagation for experiment one.

Product	Mass (kg)	%N (Std Dev)	(mass*%(N/100)) ²	Mass (kg)	% C (Std Dev)	(mass*%(C/100)) ²
Manure	4.07	0.1857	5.7123E-05	4.07	0.2526	1.057E-04
Biooil	0.838	0.0011	8.5003E-11	0.838	0.0054	2.048E-09
Biochar	1.14	0.0577	4.3267E-07	1.14	0.2535	8.3515E-06
Mass Balance N Error (δ N, kg)			0.0075	Mass Balance C Error (δ C, kg)		
				0.0106		

Table S2b) Mass balance error propagation for experiment two.

Time (min)	Exhaust Flow (m³ min⁻¹)	Exhaust (mL min⁻¹)
0.00	0.00034	340.20
12.67	0.00028	276.81
23.66	0.00022	224.95
35.00	0.00029	289.00
48.00	0.00036	362.44
66.47	0.00042	421.98
82.01	0.00047	472.08
92.01	0.00038	376.48
100.01	0.00030	300.00
108.01	0.00030	296.25
115.34	0.00029	289.06
121.34	0.00029	290.00
127.67	0.00028	284.66
135.02	0.00028	278.46
140.37	0.00027	273.94
148.67	0.00027	266.93
155.68	0.00026	261.03
162.68	0.00026	255.12
Average	0.00031	308.85
Std Dev	0.00006	62.77

Table S3: Linear interpolation used during flux calculations to account for flow instability.

Gas	CO	CO ₂	CH ₄	NO	N ₂ O	NH ₃
Slope Range (ppmv/hr)	1-13933	0-39017	0.1-2332.3	0 - 19.15	0-4.11	0-7111.2
Slope Uncertainty Range (ppmv/hr)	0-0.15	0.20-4.22	0.0-2.83	0.37-4.95	4.9E-6-4.1E-4	0.05-2.1
% Uncertainty	0-0.01	0-0.02	0-0.16	2.66-46.58	0-0.06	0-0.08

Table S4a) Range of slope estimates, range of slope uncertainty estimates (δb) and the percent uncertainty based on the range of slopes and slope uncertainty estimates for Low Temp (exp 1). While NO has a large range in %Uncertainty, the majority of measurements have an uncertainty less than 20% with a median of 13.23%.

Gas	CO	CO ₂	CH ₄	NO	NH ₃
Slope Range (ppmv/hr)	48 - 94624	40 - 102210	4 - 11534	0.0097 - 51.32	0 - 6865.7
Slope Uncertainty Range (ppmv/hr)	0.0014-4.92	0.043-12.34	0 -1.98	0-0.002	0.02-0.20
% Uncertainty	0-0.04	0-0.11	0-0.03	0-0.014	0-0.0018

Table S4b) Range of slope estimates, range of slope uncertainty estimates (δb) and the percent uncertainty based on the range of slopes and slope uncertainty estimates for High Temp (exp 2). Note N₂O was near ambient concentrations for this experiment and therefore not included in these calculations.

Gas	F (C or N kg/min)	ΔT (min)	M (kg)	δF (%)	δM (kg)
NO	5.66E-10	110	3.53E-05	0.05	1.76E-06
N ₂ O	7.97E-11	110	4.97E-06	0.01	4.97E-08
NH ₃	1.85E-07	110	1.15E-02	0.2	2.30E-03
CH ₄	7.18E-08	110	4.47E-03	0.01	4.47E-05
CO ₂	1.02E-06	110	6.38E-02	0.01	6.38E-04
CO	3.92E-07	110	2.44E-02	0.01	2.44E-04

Table S5a) Error calculation used for gas flux error propagation where F is the average slipstream emission rate for each gas as kg of N or C per min, ΔT is the total experiment time, M is the estimated total mass emitted ($F \cdot \Delta T \cdot \text{ratio of the slipstream:full flow}$) based on the average slipstream emission rate. δF is the precision uncertainty (%) of each analyzer used to measure gas emissions. The error in the mass emitted (δM) is calculated via $M \cdot \delta F$. *Table a* values are from low temp (experiment 1).

Gas	F (C or N kg/min)	ΔT (min)	M (kg)	δF (%)	δM (kg)
NO	1.16E-09	159	5.57E-05	0.05	2.78E-06
NH ₃	1.67E-07	159	8.00E-03	0.2	1.60E-03
CH ₄	2.74E-07	159	1.31E-02	0.01	1.31E-04
CO ₂	2.63E-06	159	1.26E-01	0.01	1.26E-03
CO	1.67E-06	159	8.03E-02	0.01	8.03E-04

Table S5b) Error calculation used for gas flux error propagation for high temp (experiment 2).

M $\pm$ δM (kg)		
Gas	Exp 1	Exp 2
NO	3.53E-05 $\pm$ 1.76E-06	5.57E-05 $\pm$ 2.78E-06
N ₂ O	4.97E-06 $\pm$ 4.97E-08	-
NH ₃	1.15E-02 $\pm$ 2.30E-03	8.00E-03 $\pm$ 1.60E-03
CH ₄	4.47E-03 $\pm$ 4.47E-05	1.31E-02 $\pm$ 1.31E-04
CO ₂	6.38E-02 $\pm$ 6.38E-04	1.26E-01 $\pm$ 1.26E-03
CO	2.44E-02 $\pm$ 2.44E-04	8.03E-02 $\pm$ 8.03E-04

Table S5c) Summary table of calculated mass emitted (M) using average experimental emission rate $\pm$ the error associated with that mass (δM) for both experiments.

Error Propagation (between methods)	Exp 1	Exp 2
NITROGEN		
Mass Balance N Error (δN, kg)	0.0065	0.0075
Gas Flux N Error (δN, kg)	0.0023	0.0016
Total N Error for GF and MB (δTN, kg)	0.0069	0.0077
CARBON		
Mass Balance C Error (δC, kg)	0.0158	0.0106
Gas Flux C Error (δC, kg)	0.0006	0.0015
Total C Error for GF and MB (δTC, kg)	0.0158	0.0107

Table S6: Error propagation to calculate the uncertainty in N and C based on both methods used (mass balance vs gas flux) for each experiment. Mass balance values come from δN and δC calculated in Table S2. Gas flux δN and δC values were calculated using equation 9 in the text and the δMs calculated in Table S5 for each species of N and C to propagate the total N and C error for this method.

Total Mass Emitted				
Species Measured	Exp 1 (kg)	Exp 1 (kg/day)	Exp 2 (kg)	Exp 2 (kg/day)
CO ₂ -C	5.24E-02	6.85E-01	1.19E-01	1.07
CH ₄ -C	4.15E-03	5.44E-02	1.20E-02	1.09E-01
CO-C	2.31E-02	3.02E-01	7.32E-02	6.63E-01
NO-N	3.33E-05	4.36E-04	5.32E-05	4.82E-04
NH ₃ -N	9.67E-03	1.27E-01	7.76E-03	7.03E-02
N ₂ O-N	7.38E-06	9.66E-05	-	-

Table S7: Total C or N mass emitted of all carbon and nitrogen species measured (kg C or N) over 110 minutes (experiment 1) or 159 minutes (experiment 2) and emission rates (kg/day) for each experiment.

Mass Loss by Method	Exp 1	Exp 2
MB C+N mass lost (kg)	0.614	0.682
GF C+N mass lost (kg)	0.089	0.212
<i>difference</i>	0.525	0.470
MB total C mass lost (kg)	0.562	0.623
GF total C mass lost (kg)	0.080	0.204
<i>difference</i>	0.482	0.419
MB total N mass lost (kg)	0.052	0.059
GF total N mass lost (kg)	0.010	0.008
<i>difference</i>	0.042	0.051

Table S8: Calculated total masses (kg) of N and C by mass balance (MB) and gas flux (GF) and the differences in mass by approach for experiments one and two.

Scaling up NH ₃ Emissions within Chesapeake Bay Watershed	
NH ₃ emission rate (our measurement, kg/kg)	1.4 x 10 ⁻³
Manure applied (kg/yr) ¹⁴	8.64 x 10 ⁹
Possible NH ₃ emitted (kg)	1.2 x 10⁷
%N (Manure - our measurement)	2.69
Manure Moisture Content	0.275
Dry Manure (kg)	6,264,000,000
Manure N Mass (kg)	168,501,600
Percent Loss as NH ₃ (low estimate)	0.2
Percent Loss as NH ₃ (high estimate)	0.6
NH ₃ Loss from Total Manure N applied (low, kg)	3.4 x 10⁷
NH ₃ Loss from Total Manure N applied (high, kg)	1.0 x 10⁸
Pyrolysis Loss Vs Land Application (low, %)	35
Pyrolysis Loss Vs Land Application (high, %)	12

Table S9: Scaling up our measurements, the calculations in the table above emulate a typical working production schedule at the USDA where we conducted our measurements. The NH₃ emission rate of 0.00776 kg per 159 min can be divided by the total mass (5.6 kg) to obtain an emission rate of 1.4 x 10⁻³ kg NH₃-N per kg of manure via pyrolysis. Using the total manure applied annually in the Chesapeake Bay Watershed¹⁴, an estimate for the total amount of NH₃ emitted in kg can be quantified by multiplying the emission rate by the total manure applied annually. Assuming the same manure moisture content and manure %N as in our mass balance calculations, the dry manure mass and N mass of that manure can be calculated by: Manure Mass*(1-Moisture Content) and Dry Manure*(%N/100). Finally, as much as 20-60% of total N applied is emitted as NH₃. This was calculated by quantifying the product of Manure N mass*0.2 and Manure N mass*0.6 to obtain the range of possible NH₃ emissions from chicken manure soil applications. Pyrolysis Loss vs Land Application is simply the ratio of pyrolysis NH₃ emissions to NH₃ emitted from soil applications to calculate the proportional difference.

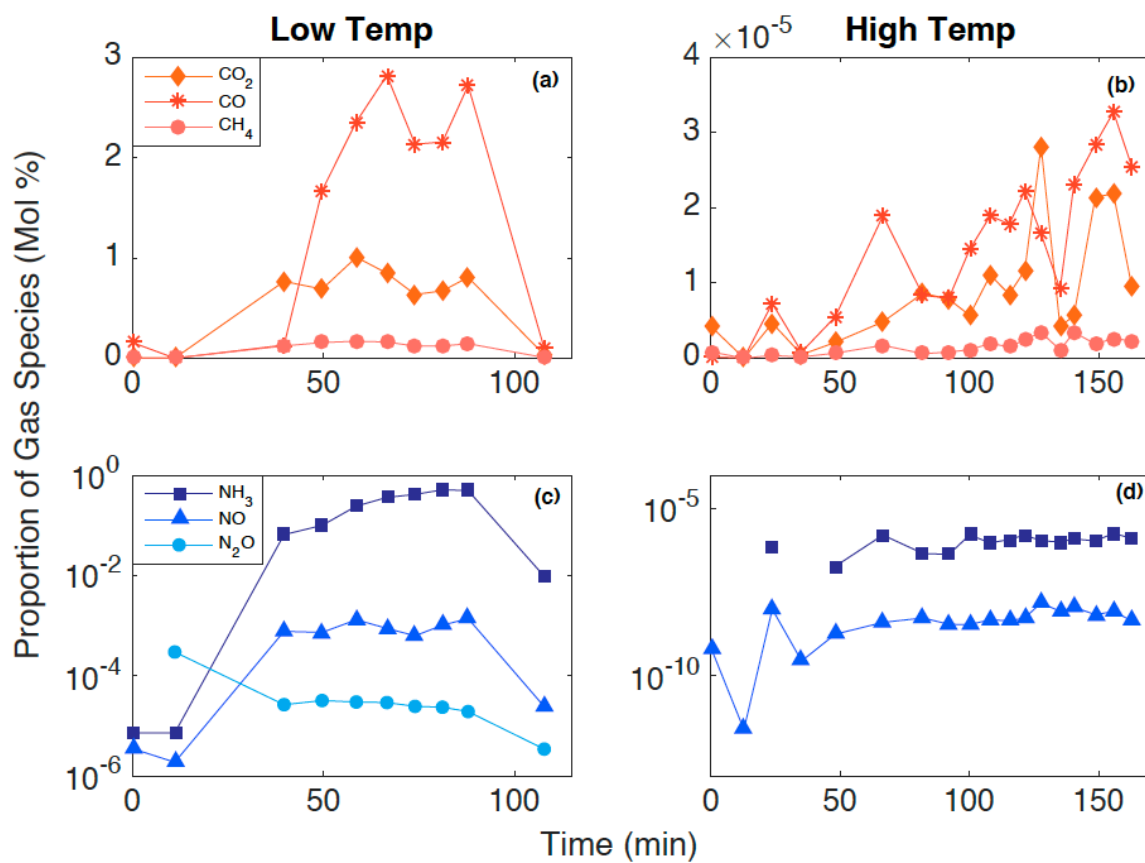
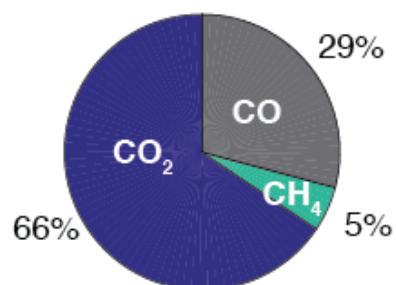


Figure S1: Proportions of each gaseous species (mole percent), for experiment 1 (left) and experiment 2 (right). Data is presented on a semi-log plot for better visualization for all nitrogen species. (N₂O is not included in the second experiment as it was not detectable above ambient concentrations.) Note the difference in scales.

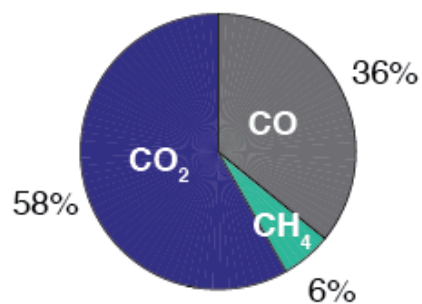
% C Species at Low Temp



% N Species at Low Temp



% C Species at High Temp



% N Species at High Temp

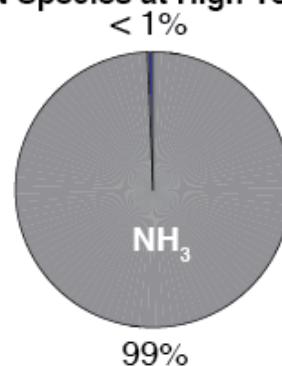


Figure S2: Gas emission percent composition of C and N species at low (exp 1) and high (exp 2) temperature conditions. The <1% for N species is for NO and N₂O (exp 1) and NO (exp 2).

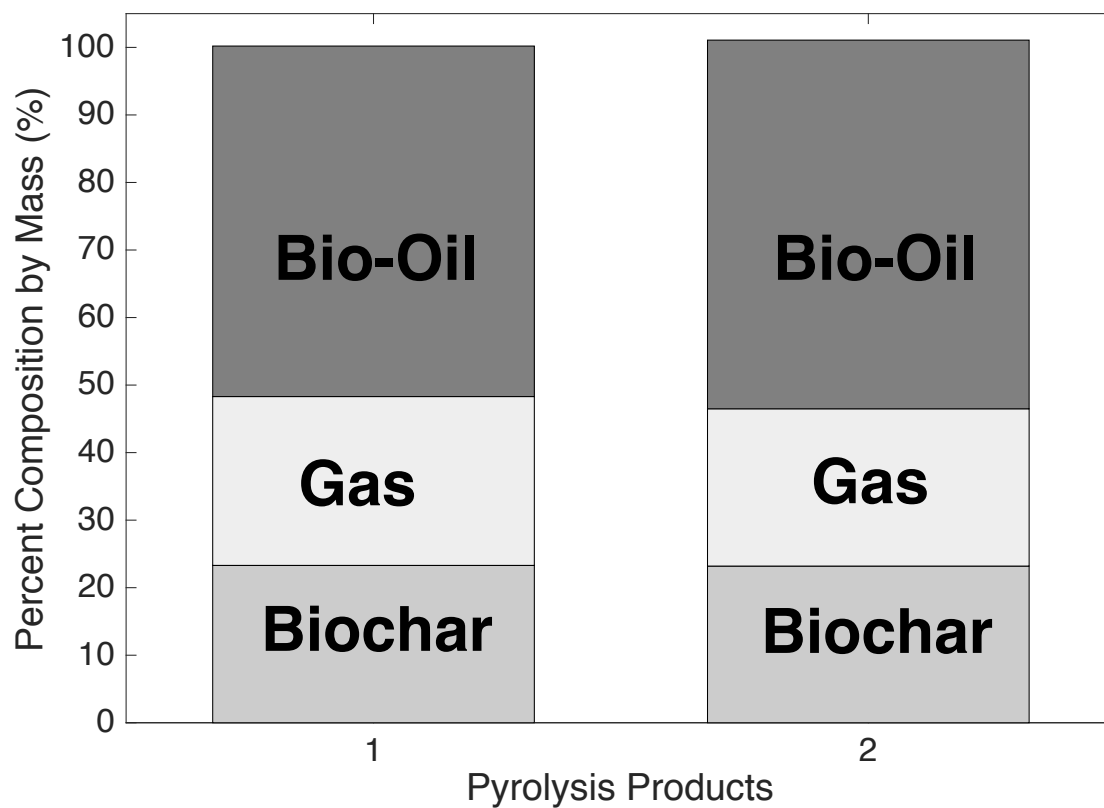


Figure S3: Stacked bar plots of pyrolysis products: biochar, gases and bio-oil by percent mass composition during experiment 1 (left) and 2 (right).

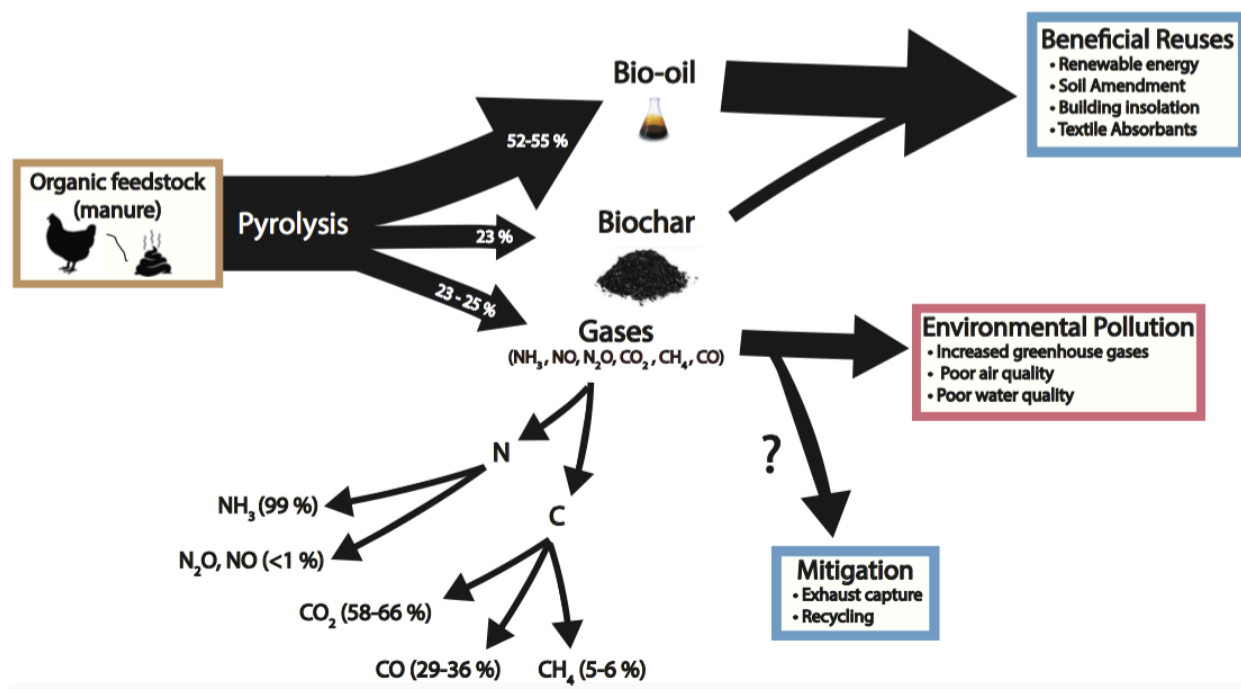


Figure S4: Summary figure of pyrolysis experiments conducted in this study using chicken manure as the feedstock. Pyrolysis arrows describe the percent composition by mass of each pyrolysis co-product produced from the original feedstock. Some of which have beneficial reuses or are associated with environmental pollution and have possible mitigation strategies. Gases are broken down into their C and N components and characterized by percent composition of each species measured.

Appendix Two

Changing policy: how has additional sewage treatment altered
N and C biogeochemistry in a historically eutrophic system?

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Preamble:

New England's largest estuary, Narragansett Bay, has a history of poor water quality due to the onset of industrial activity and sewage effluent management into the Bay, releasing a lot of nitrogen into this system. Extensive management efforts have been made to reduce anthropogenic nitrogen nutrient loads (especially from sewage treatment facilities) to minimize its impact on the ecological stability of the Bay and assess whether these mitigation efforts have been effective. However, despite major reductions (~60%) in N loads from wastewater treatment facilities in Rhode Island, Narragansett Bay is still heavily influenced by sewage N loads and acts as an important food source for portions of the Bay (Oczkowski et al., 2018). In 2016, we collected two sediment cores in Narragansett Bay downstream from one of the wastewater treatment facilities to assess whether amendments in wastewater treatment practices were evident in estuarine sediments using bulk $\delta^{15}\text{N}$. The enrichment in $\delta^{15}\text{N}$ we observe aligns with a recently proposed theoretical model supporting the hypothesis that sewage N is still an important nutrient source (Oczkowski et al., 2018, see Figure 10). We present isotopic evidence that indicates estuarine sediments fit this theoretical model, which has implications for the successful implementation of mitigation strategies with respect to wastewater management and ecosystem response. Our intention is to prepare this work for peer-reviewed publication as a short and punchy "proof of concept" in a journal such as *Scientific Reports*. The proceeding text consists of sediment core analyses of metal concentrations (lead, zinc, copper), carbon and nitrogen sedimentary content and stable isotopic composition in the 2016 cores and complementary cores within the estuary to tease apart patterns in N and C biogeochemistry. This work is in a preliminary state and will change considerably prior to publication (except for the data). It is included here to document these efforts and to illustrate the broader aspects of my N studies.

1. Introduction

Nitrogen is an essential macronutrient to primary production that limits growth in most marine systems. It is made bioavailable through N_2 -fixation, and the supply of bioavailable nitrogen has been dramatically altered due to anthropogenic activities, predominantly fossil fuel combustion, agricultural fertilizer use, and sewage discharge. These activities have perturbed the natural nitrogen biogeochemistry in many ecosystems causing nitrogen saturation on land, which has negative cascading effects such as soil and water acidification, eutrophication and poor air quality (Fowler et al., 2013). This cycle is commonly referred to as the Nitrogen Cascade (Galloway et al., 2003) and has been at the forefront of environmental changes in many ecosystems. In particular, water bodies located near heavily urbanized areas and trading/shipping ports suffer from nitrogen discharged from industrial activity, urban development, and sewage infrastructure that contribute to this problem.

Narragansett Bay is a large estuary off the coast of Rhode Island that has a long history of poor water quality and habitat degradation as a result of industrialization and sewage management (Nixon, 1997; Bricker, 1993). Since the early 20th century, sewage effluent has been a major input of nutrients into the bay and dominated the nitrogen-loading budget. Marine and estuarine habitats are typically thought to be N-limited, and sensitive to any extraneous nitrogen inputs (Howard and Marine, 2006). This idea is supported by previous work in lower sections of Narragansett Bay that are proximal to the open ocean (Fulweiler et al., 2007; Nixon, 1997). However, the upper part of Narragansett Bay is not nitrogen-limited. These works ignore the full extent of estuarine water that penetrates the northern Rhode Island coastline, which disregards the upper portion of the bay that contributes to high nutrient loading. This upper section includes the Providence and Seekonk Rivers, which deliver large supplies of nutrients

from wastewater treatment facilities, surface storm-water runoff, power plants etc. As a result, it is important to delineate the bay into upper and lower sections, where water closest to Providence reflects a nitrogen saturated habitat and water south of Prudence Island is more nitrogen limited. As a result, a host of ecosystem problems including eutrophication, low dissolved oxygen and fish kills have had an impact on the upper ecosystem over the course of many decades, particularly in regions closest to urban activities of Providence. The consequences of many of these environmental issues have a direct impact on recreational (public use) and socio-economic (fisheries, shipping) ecosystem services to the region.

In 2012, tertiary treatment at wastewater treatment facilities in the Narragansett Bay watershed was introduced as an additional step to reduce the nitrogen and phosphorus pollution discharged into the bay, which previously only used primary and secondary treatment phases. Prior to tertiary treatment, nitrogen is typically present as ammonia, but this additional step allows for the conversion of ammonia via nitrification and denitrification to non-toxic nitrate and dinitrogen gas (Naidoo and Olaniran, 2014). Since initial policy mitigation was enacted to monitor and cut effluent, pollution has been cut by 60% since the 1970s (Save the Bay, 2000), which has improved the overall health of the bay, including the opening of recreational beaches in recent years previously closed due to pollution. However, water quality in many parts of the bay still represents a major health hazard, unsuitable for swimming, and low oxygen events often induce anoxic conditions and fish kills. An additional challenge involves the wastewater treatment facilities located in Massachusetts, which discharge into the Narragansett Bay watershed, but fall under different jurisdictions. As Duarte et al., (2009) suggests, baseline or steady state conditions can be challenging to return to in a timely manner after an ecosystem experiences a perturbation. A 60% reduction in overall effluent over the span of four decades

might improve the overall nutrient imbalance experienced by the nutrient budget, but it is unclear how this will continue to affect the bay and whether eutrophic conditions can ever be stopped entirely.

The Bucklin Point wastewater treatment facility is one of nineteen facilities that discharge pretreated effluent into waters that flow into Narragansett Bay. Located on the Seekonk River, it presents an opportunity to observe and assess temporal trends in nitrogen and carbon biogeochemistry preserved in sedimentary records since the introduction of tertiary treatment. Using a recently sampled sedimentary record from the Seekonk River, my objective is to assess the sensitivity of nitrogen and carbon biogeochemical cycles within the northern reach of the Narragansett Bay Watershed to changes in wastewater treatment that have taken place from 2012 to present. Particular attention will be paid to nitrogen since it is a driving component of nutrient loading and eutrophication in this system. This is important if we are to understand ecosystem sensitivity to nutrient imbalances associated with anthropogenic activities and sources.

Questions

- 1) How has the 2012 implementation of tertiary treatment at wastewater treatment facilities (WWTF) influenced nitrogen cycling/biogeochemistry within sediments downstream from WWTF discharges in the Narragansett Bay Estuary?
- 2) Have any shifts in carbon biogeochemistry occurred that suggest a change in productivity level?

Hypotheses

- 1) Sediments immediately downstream of the WWTF will preserve an enriched isotopic nitrogen signature in the top ~10 cm. The implication of an enriched signal is that the

oxygen conditions are minimized from an influx of nutrients, such that denitrification is the major oxidation process of organic matter, potentially resulting in more N₂O gas production.

- 2) While decreases in sewage effluent have improved areas around the bay, productivity levels will not have changed drastically over a four-year period due to additional nutrient inputs from surficial runoff, atmospheric deposition, and WWTF discharges from Massachusetts facilities.

2. Methods

Chemical stratigraphy was used to date a short core sampled in the Seekonk River using a couple of previously sampled cores from a 2010-2012 transect (Cores 71, 72 and 73). Biogeochemical patterns in nitrogen and carbon were analyzed using stable isotopes, total abundance and inorganic and organic C with particular emphasis on nitrogen.

2.1 Bucklin Point Core Collection

Our intention was to collect surficial sediments that would represent sediment accumulation over the past four years to capture the sedimentary history since tertiary treatment was implemented. Sedimentation rates are quite variable in the Seekonk River given its history as a shipping channel, but based on previous work (Prell, personal communication), we estimated that the top ten centimeters would cover a range of sedimentation rates in the area that reflect the last four years of sediment accumulation. In March 2016, two short cores (36 and 45 cm in length) were collected in the Seekonk River just downstream from the Bucklin Point Wastewater Treatment Facility and south of Swan Point. The 2016 cores are referred as Clark71. Each core was sub-sectioned accordingly for cores one and two (0.5 cm increments in the top 10

cm followed by 2 cm increments; 1 cm increments in the top 10 cm; followed by 2 cm increments). All samples were freeze-dried for 24-48 hours and finely homogenized. Samples were not acidified. Previous work (Murray, D. et al. unpublished data) found CaCO_3 to be negligible within Seekonk sediments, but more prominent as you move further south in Narragansett Bay. As a result, it was deemed appropriate to leave samples non-acidified for all analyses. The elemental/chemical composition and magnetic susceptibility were measured using x-ray fluorescence (XRF) and a magnetic susceptibility meter. Reflectance was not measured.

2.2 Seekonk River Transect: XRF Cross Correlations

A transect of surficial sediment cores were collected in the Seekonk River in the summers of 2010, 2011 and 2012 by Warren Prell, Dave Murray and Joseph Orchard. These cores were used to explore the spatial and temporal trends in metal composition, sedimentation rates and isotopic composition of the Seekonk River as a way to characterize trends in the upper section of Narragansett Bay. All cores were measured for elemental and chemical composition, and magnetic susceptibility using XRF. Cores NB71, NB72 and NB73 were all collected in 2012 and are closest to the collection site of the Clark71 cores. Of these three NB records, only NB71 was analyzed for total carbon and nitrogen (TC, TN) and stable isotopes of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Unfortunately, none of these cores were dated using ^{210}Pb . The Seekonk River has a suite of unique sedimentation zones, where sedimentation and accumulation rates vary depending on the orientation from the channel, which are represented by the sampled cores taken during this field campaign. X-ray fluorescence data was used to determine correlations between elemental profiles of lead, zinc and copper concentrations between 2012 cores: NB71, NB72, NB73 and the Clark71 cores, which were sampled close to the original NB 71 sample site (Table 1). These metals are helpful tools for dating a relative age of the sediment. Lead is a useful

sediment dating tool to determine the peak and sharp decline of leaded gas use in the 1970s. Copper is useful for dating World War II because jewelry production ceased during this period decreasing concentrations of this metal within sediments. All three of these metals are useful for dating the beginning of the industrial revolution in 1850. These events have consistently been found within sedimentary records and effective diagnostics for age estimates. Given, the length of the 2016 cores, it is unlikely that a pre-industrial signal would be present. However, elemental concentrations of Pb, Cu and Zn can be used to make inferences about similar features among cores that correspond to age estimates. If correlations can be inferred from the metal profiles and associated sedimentation rates between core sets, then it may be possible to get a reasonable age estimate for the 2016 core, which can be applied to an age-depth model, and verify how many centimeters of sediment represents the tertiary treatment period (2012-2016) based on previously measured sedimentation rates in the area.

Core	Latitude	Longitude	Length(m)	C/N	XRF	¹³ C/ ¹⁵ N	Description of Site
NB71	41.8478	71.37635	0.89	X	X	X	Swan Point transect west
NB72	41.84705	71.36996	0.96		X		Swan Point transect east (Bucklin)
NB73	41.84826	71.37502	1.13		X		Swan Point transect center
Clark71_1	41.846	71.383	0.45	X	X	X	Swan Point transect west
Clark71_2	41.846	71.383	0.36	X	X	X	Swan Point transect west

Table 1: Description of cores collection sites, core length, data available for each core and a description of the site.

2.3 Elemental analysis: C and N total abundance

Elemental analysis of C and N was conducted to obtain total abundances of nitrogen and carbon to look at inorganic and organic components in Clark71. The ratio of C:N was derived from these abundance measurements, which provides useful information about the

sources/origins of organic matter. In addition, this data was compared to the C and N data available for the Seekonk records collected in 2012 to see how both records align and what changes may have occurred. Abundance data was only available for NB71 to make comparisons with the Clark71 record.

2.4 Inorganic Carbon

Inorganic carbon was measured using coulometry to determine the presence or absence of inorganic carbon that may interfere with interpretations of the $\delta^{13}\text{C}$ isotope data. Using the inorganic carbon measurement, the difference between total C and inorganic forms were calculated to obtain a value for the organic portion. If inorganic carbon is present, the interpretation of the carbon isotope and potentially, the nitrogen isotope record could be compromised without acidifying and rerunning samples for bulk C and N.

2.5 Bulk stable isotopic analysis: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Bulk stable isotopes of C and N have been quantified and were used to interpret depositional history and changes to biogeochemical cycling with an emphasis on nitrogen in this low, oxygen reducing environment. Nitrogen isotopes can relay information regarding recycling pathways utilized in the sediments during organic matter decomposition in oxygenated and oxygen-limited environments. Denitrification is a dominant metabolic pathway where nitrate is the favored terminal electron acceptor during the breakdown of organic matter in anaerobic environments. This pathway is common in nutrient-saturated environments due to the magnitude of primary production and oxygen consumption during respiration and is reflected by an enriched isotopic signature (Tesdal et al., 2012). Bacterial processes, such as denitrification and nitrification, induce large fractionation effects that can be as high as -25 ‰ (Brandes et al., 1998) and leaves the remaining substrate enriched in ^{15}N with typical ranges between 10 to 15 ‰

(Kritee et al., 2012). However, additional environmental characteristics, including oxygen availability enable the disentanglement of these two processes. The presence of a positive $\delta^{15}\text{N}$ is anticipated during the 2012-2016 period within the Clark71 record.

Carbon isotopes provide information regarding how ecosystem productivity changes over time (O'Reilly et al., 2003). While acidification of the sediment was not conducted, CaCO_3 has been found to be a minor component of the sedimentary record in the Seekonk River to be concerned with its effects on the interpretation of carbon isotope signatures (Murray et al., unpublished data). This may be a result of CaCO_3 dissolution in acidic soils common in this environment. Prior to interpreting any isotopic values of carbon, total abundance, inorganic and organic carbon was quantified to verify the presence of inorganic carbon. The organic portion was quantified by taking the difference between the total abundance and inorganic portions. If inorganic carbon is present, the isotopic record for ^{13}C will not be interpreted to avoid false interpretations of past productivity. O'Leary (1981) recognized that $\delta^{13}\text{C}$ could be used to distinguish different photosynthetic pathways, where C_4 plants exhibit values around -12‰ and typical $\delta^{13}\text{C}$ for C_3 plants range from -24 to -30‰. Estuarine phytoplankton have typical $\delta^{13}\text{C}$ values that range from -35 to -20‰ (Bouillon et al., 2011). Knowledge of these pathway differences can reflect changes in ecosystem productivity where lighter or more negative values reflect less productive systems and heavier or less negative values reflect high productivity levels. This is due to ~20‰ isotope effect during photosynthesis and assimilation of CO_2 in organic matter (O'Leary, 1988). In addition, the fraction of inorganic and organic carbon components can be calculated using a set isotopic composition for organic and inorganic carbon based on known isotopic ranges for each end-member. Equation (1) was used to conduct this calculation, where f is the percent of organic carbon and $1-f$ is the percent of inorganic carbon

needed to acquire the isotopic change recorded in the surface sediments. The isotopic composition for organic carbon was set to -26 ‰, inorganic carbon was set to 0 ‰ and the measured isotopic composition of bulk C from the cores was used for the total C.

$$(1) \text{ Total } C = \text{organic } C (1-f) + \text{inorganic } C (f)$$

3. Results

3.1 XRF Cross Correlations

Cross correlations for zinc, copper and lead were most useful for making comparisons between Clark71 and NB71 because they have similar structural integrity and sedimentation rate, which is logical since they were sampled close to one another. Copper was used to draw ten initial correlations between concentration and depth then linear interpolation was used to align both cores (Figure 1). In both copper and lead, a rise in concentration occurs at ~55-60, 50 and 15 cm (Figure 1, panels 1 and 2). For copper, concentration ranges between the two cores are: ~100-150 ppm, 650-810 ppm and 600-800 ppm, respectively for the depths mentioned above (Figure 1). Lead concentration ranges at each of these depths are: ~50-200 ppm, 200-220 ppm and 250-310 ppm (Figure 1). There are less distinguishable peak features in the zinc records, but there is a distinct increase in concentration levels (50-100 ppm) that correspond with industrial activity (Figure 1). When these same correlations are made with zinc and lead, some similarity in structure is apparent, but a more robust dating method and additional correlations are needed to construct a better age model. Direct correlations between NB71, NB72 and NB73 were challenging due to different sedimentation rates and less distinguishable concentration features. NB73 appears to have a higher sedimentation rate than NB71, such that the sediments represented within the collected core do not contain a pre-industrial signal (Figure 2). While

concentration levels were comparable in NB71 and NB72 for zinc, copper and lead, concrete correlations could not easily be distinguished (Figure 3). These discrepancies complicated the age-depth correlations using XRF data without additional dating techniques.

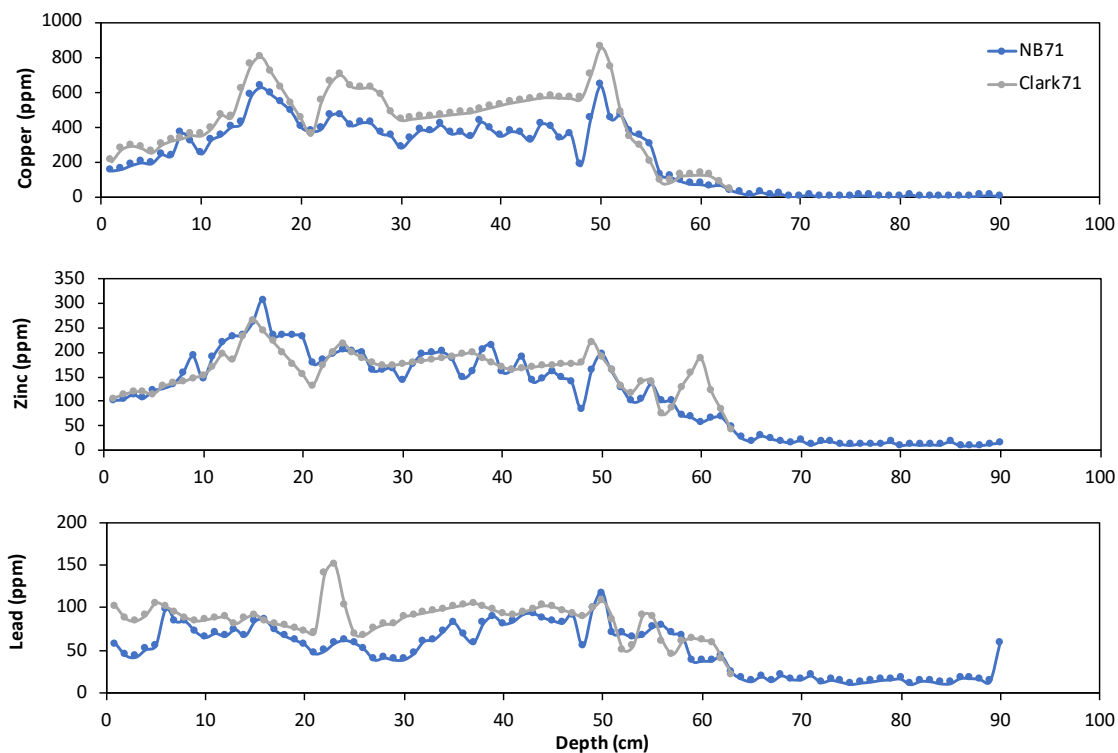


Figure 1: XRF elemental compositions for copper, lead and zinc for cores NB71 and Clark71. NB71 is represented by blue circles. Clark71 is represented by grey circles.

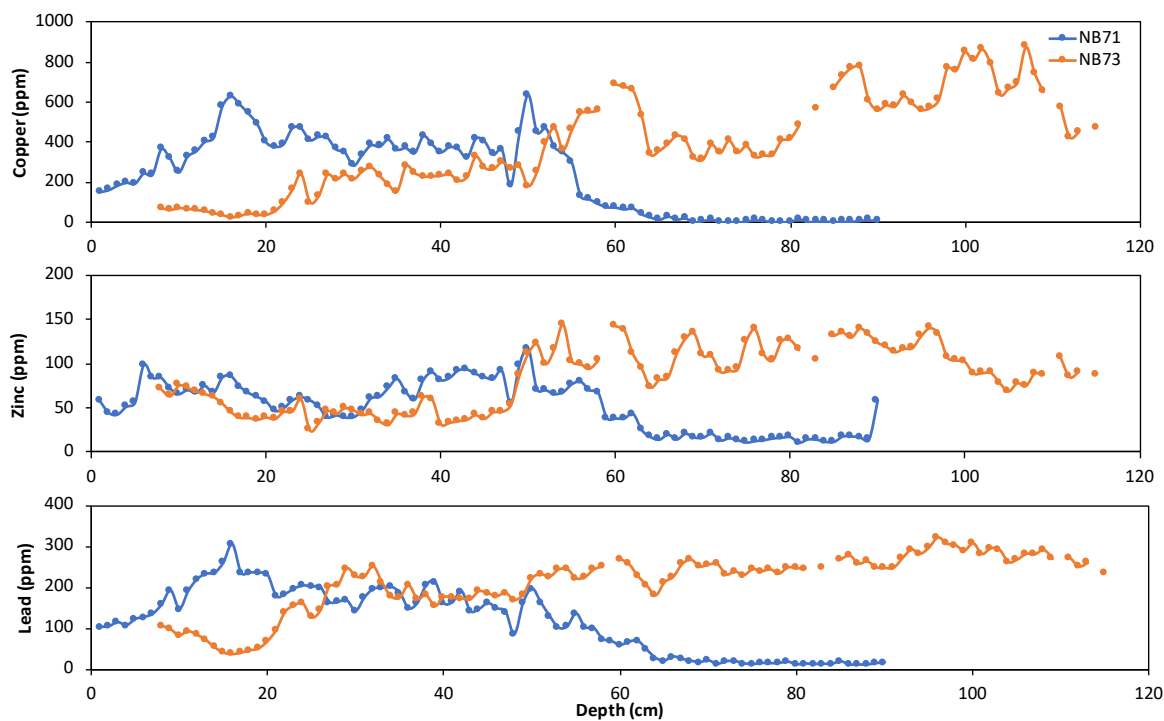


Figure 2: XRF elemental compositions for copper, lead and zinc for cores NB71 and NB73. NB71 is represented by blue circles and NB73 is represented by orange circles.

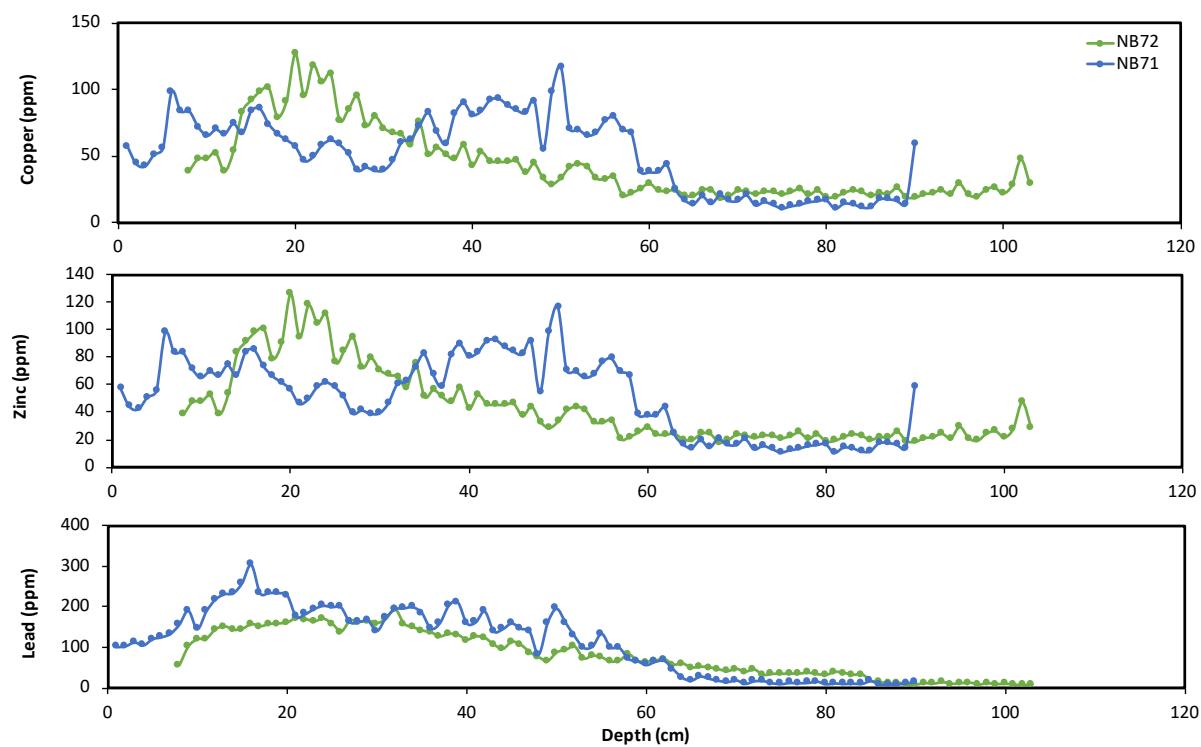


Figure 3: XRF elemental compositions for copper, lead and zinc for cores NB71 and NB72. NB71 is represented by blue circles. NB72 is represented by green circles.

Unfortunately, none of these cores (NB71, NB72, NB73) have been dated using ^{210}Pb and have dynamic sedimentation rates presenting challenges for making cross correlations and effectively dating Clark71. However, a depth-age scale was estimated using lead and copper concentration profiles for both cores: Clark 71 and NB 71 (Figure 4). Age estimates included on the scale consist of the beginning of the industrial revolution in 1850, the Clean Air Act and demise of leaded gasoline in 1973, 2012 and 2016 for the two recent collection dates (Figure 4).

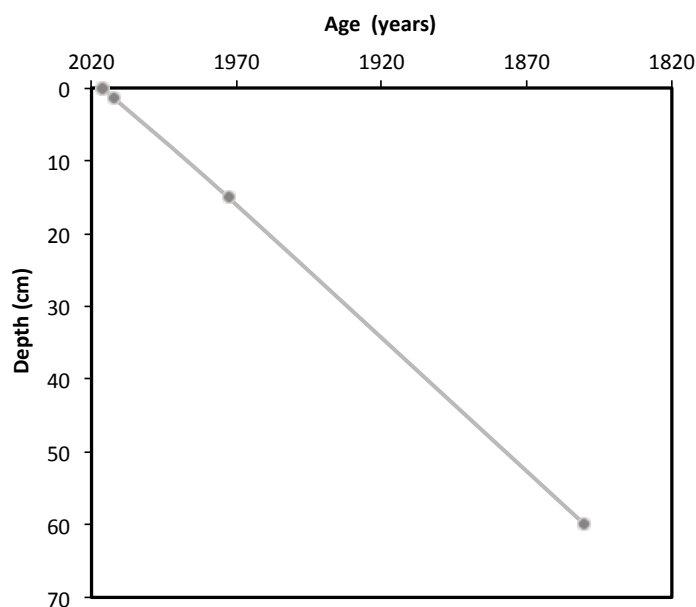


Figure 4: Age-Depth model using elemental concentration data for copper, zinc and lead. Age estimates included on this scale include: 2016, 2012, 1973 and 1850.

3.2 Elemental analysis: C and N total abundance

Elemental analyses were conducted for both Clark 71 and NB 71. Unfortunately, NB 72 and NB 73 do not have total abundance data available to include in this analysis. Percent carbon and nitrogen exhibit increasing trends that range from 0.37-12.94% and 0.01-0.57%, respectively for NB 71 (Figure 5). Clark 71 had percent C values that ranged from 2.66-10.54% and percent N values that ranged from 0.17-0.57% (Figure 5). Clark 71 exhibited total carbon values that ranged from 26.40 – 92.69 mg C, while total nitrogen ranged from 1.71-5.69 mg N (Figure 5).

Similarly, total C ranged from 3.26-112.44 mg and total N ranged from 0.13-5.13 mg for NB 71 (Figure 5). While these values fluctuate throughout the cores, there is a general increasing trend in total C and total N from the bottom to the top of the core. C:N ratios range from 15.49-39.40 in NB 71 while Clark 71 C:N ratios range from 13.31-20.46 (Figure 6). In both cores, C:N ratios remain stable between 15-20 in the most recent past (~5 cm) (Figure 6). These trends in carbon and nitrogen correlate with nutrient inputs from anthropogenic activity and land use changes.

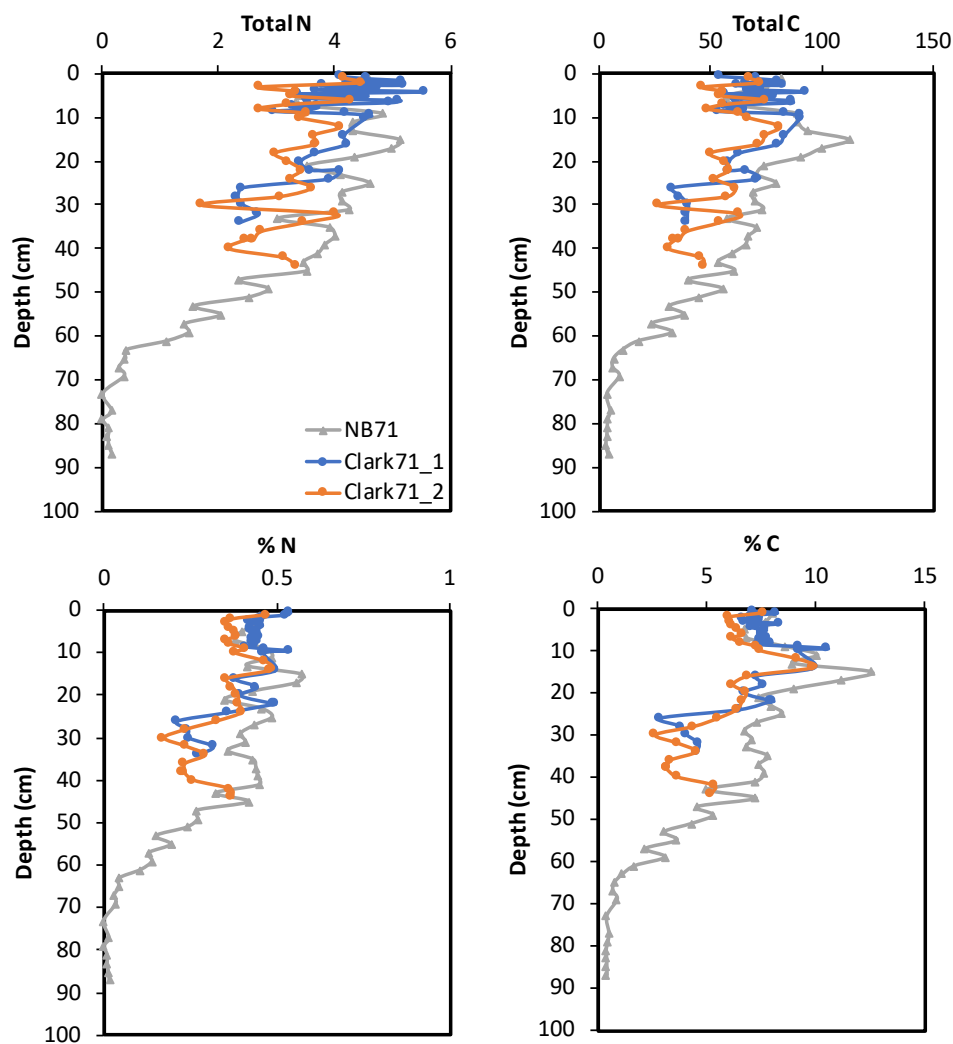


Figure 5: Four panel plot from left to right: 1) Total N, 2) Total C, 3) %N, 4) %C. All components are plotted against depth. NB71 is represented by grey triangles, while Clark71, cores 1 and 2 are represented by blue and orange circles.

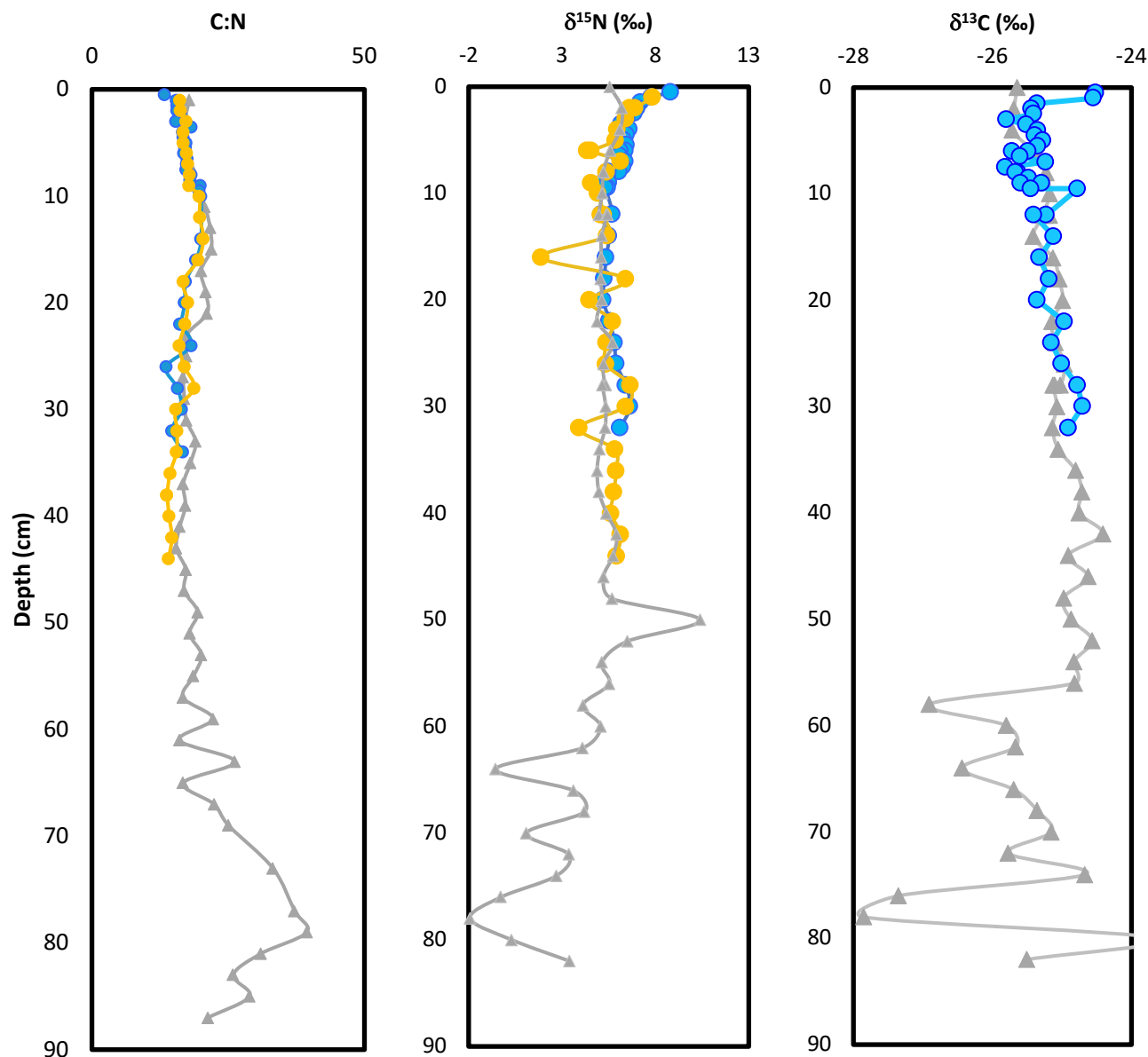


Figure 6: Three panel plot of C:N, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ versus depth. Grey triangles represent NB71. Blue circles represent Clark71, core 1 and yellow circles represent Clark71, core 2.

3.3 Inorganic Carbon

Of the 19 samples measured for inorganic carbon in Clark71, all exhibited very low/trace amounts of inorganic carbon near the top and bottom core sections. Low inorganic carbon suggests that the interpretation of the bulk C and N isotopic signatures will not be compromised due to the presence of carbonate in the sediments, because minimal carbonate appears to be

present within the record. Organic carbon values ranged from ~26,000-82,000 ppm while inorganic carbon values were as little as ~17-284 ppm. Organic carbon is the dominant form of carbon present within these sediment samples and comprises as much as 99% of the total carbon content in the majority of samples.

3.4 Bulk stable isotopic analysis: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

$\delta^{13}\text{C}$ values range from -26 to -24 ‰ while $\delta^{15}\text{N}$ values range from 5 to 8.8 ‰ in Clark71 (Figure 6). In contrast, the $\delta^{13}\text{C}$ values range from -28 to -24.4‰ while $\delta^{15}\text{N}$ values range from 4.1 to 6.5 ‰ in NB71 (Figure 6). Below 50 cm, NB71 exhibits a depleted signal in both isotopes, which may reflect changes in source loading and productivity pathways. Above 50 cm, NB71 has comparable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with Clark71 (described above). However, in the top few centimeters of surface sediment, the cores deviate by ~1-2 ‰ shift in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. This is evident in ~2 ‰ enrichment in ^{15}N from 6.9 to 8.8 ‰ and ~1 ‰ from -25.6 to -24.5 ‰ enrichment in ^{13}C (Figure 6). Given a low sedimentation rate of ~0.36-0.38 cm yr⁻¹, it is estimated that 1.4-1.5 cm of sediment represents the 4-year hiatus between core collections. To obtain a 1‰ change in the carbon content, 94.24% of organic carbon and 5.76% of inorganic carbon would need to be added to obtain the isotopic signature measured within the sediments.

4. Discussion

Due to its position downstream from the Bucklin Wastewater Treatment Plant, the cores collected in 2012 (NB71) and 2016 (Clark71) comprise a comprehensive view at biogeochemical changes occurring within the ecosystem since the implementation of tertiary treatment. Low in oxygen content and high in organic matter, this site promotes ideal conditions for microbial

denitrification and alterations in productivity. However, slow sedimentation rates might present challenges in visualizing changes in the sedimentary record since 2012.

While there are limitations with the current age model, the estimated range of sedimentation rates for Clark71 and NB71 include $\sim 0.36\text{-}0.38\text{ cm yr}^{-1}$ using 2016, 2012, 1973 and 1850 as reference dates. Within the last four years of sediment accumulation, $\sim 1.4\text{-}1.5\text{ cm}$ was deposited at the collection site of Clark71. Within these surficial sediments, isotopic shifts of 1-2 ‰ are evident in enriched ^{15}N and ^{13}C . These enriched isotopic signals are representative of denitrification and a slight increase in productivity levels from previous years. The implication of these findings suggests that microbial denitrification has increased with tertiary treatment and stimulates primary production. As a result, this reduces the amount of nitrate accessible within the ecosystem and increases the amount of N_2 or N_2O released into the atmosphere. While this might minimize nitrogen pollution within Narragansett Bay, this could be a potential source of greenhouse gas emissions that have consequences for climate change. However, this is contingent on what fraction is released as N_2 gas vs N_2O .

Above 50 cm, both profiles show a steady depletion in ^{13}C (-27 to -24‰), while $\delta^{15}\text{N}$ (3 to 7 ‰) is fairly stable up until the recent past (e.g. 2012-2016) where $\delta^{15}\text{N}$ becomes more positive (7 to 8.8 ‰). This may reflect the reductions made in nitrogen loading from wastewater treatment facilities prior to tertiary treatment in 2012, which have minimized nitrogen pollution by 60% (Save the Bay, 2000). However, urban runoff and atmospheric deposition still supply nutrient sources (Vadeboncoeur et al., 2010). Though unclear how large of role they play (Vadeboncoeur et al., 2010), it is likely significant, especially with changes in amount and severity of precipitation events with impending changes in regional and global climate. The depleted carbon signature might reflect a decrease in productivity or a shift in the algal

community.

Relative to carbon, nitrogen content is low in the Seekonk River (Figure 5). Total C and N levels begin increasing around the industrial revolution when anthropogenic activities began to ramp up around Narragansett Bay, which was an active shipping and trading port at the time. Around the 1970s, they reached their peak and have begun to taper off in the past few decades (Figure 5). This suggests that around 1973, a surplus of organic matter may have originated from terrestrial/anthropogenic sources reflecting peak productivity, but as nutrient loading was reduced nitrogen became more limiting and minimized primary production slightly. C:N ratios in both cores increase from 13 – 20 from the surface to 16 cm (Figure 6). Organic matter mineralization and long-term diagenesis are responsible for values closer to 20, while C:N ratios closer to 15 might reflect algal growth in more nitrogen limited water. However, it is important to remember that the upper portion of the bay is not nitrogen limited. This system is dominated by organic carbon, which makes up ~95-99% of most sediments sampled, while inorganic carbon comprises as little as 5%. As a result of microbial respiration and release of CO₂, acidic conditions may result in dissolution and poor preservation of any inorganic carbon present in Seekonk depositional environments. Due to high nutrient loading, primary production enables organic matter synthesis and CO₂ sequestration, but acidified conditions suppress inorganic carbon pools within the Seekonk River.

Before the 1970s (e.g. below 50 cm), both isotopes fluctuate toward more depleted and enriched values. The presence of low, negative $\delta^{15}\text{N}$, -2 to 1 ‰, values suggests that atmospheric deposition of nitrogen oxides from fossil fuel combustion may have had a larger influence after 1850 or that N₂-fixation was important during periods of low nitrogen availability in this system prior to intensified nutrient loading. Fluctuations in $\delta^{13}\text{C}$, -27 to -25‰, suggest that there were

natural periods of low and high productivity that relied on autochthonous sources of nutrient inputs characteristic of estuarine phytoplankton and C₃ plants. Total C and N, C:N ratios, % N and %C all reflect a steep transition from background levels of these elements within the environment to an ecosystem experiencing drastic changes due to industrialization, urban pollution and land-use changes.

In sum, the isotopic shift present in the surface sediments of Clark71 corresponds well with the implemented 2012 policy changes in wastewater treatment. The environmental response is evident within the sediment during this short time frame and microbial denitrification is taking place. It also appears that the carbon cycle is responding with a shift toward a more productive system with $\delta^{13}\text{C}$ signatures reflective of C₃ photosynthetic pathways and estuarine phytoplankton (Bouillon et al., 2011). This indicates that the environment is capable of adapting quickly after environmental perturbations, such as shifts in nutrient availability, to establish a new state of equilibrium (Duarte et al., 2009). Incorporating ^{210}Pb dates or ^{14}C values would improve our age model and the interpretation of the carbon and nitrogen data. Isotopic analysis and elemental analysis of the remaining Seekonk Cores collected in 2012 would provide additional context for interpreting changes in productivity, nutrient loading and metabolic pathways in the past. Additional cores would need to be sampled downstream from other Narragansett Bay WWTFs to validate the occurrence and role of microbial denitrification within the estuary. A comprehensive and rigorous analysis of all cores would verify whether tertiary treatment practices promote substantial off-gassing of N₂O to the atmosphere, as well as, how these changes will alter the carbon and nitrogen biogeochemistry of a region that seeks a healthy estuarine environment.

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